



**FACULTAD DE CIENCIAS
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**“ACTIVITY-DEPENDENT REGULATION OF THE GABAERGIC
SYNAPSE”**

**Thesis Project to qualify for the degree of
“Magister en Ciencias Biológicas Mención Neurociencias”.**

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ABSTRACT:

Excitatory and inhibitory balance play a fundamental role in regulation and maintenance of cerebral activity. Inhibitory regulation occurs by the action of diverse GABAergic cells that show activity-dependent changes. Here, we focus on plasticity of inhibitory synapses mediated by somatostatin-expressing interneurons (SOM-INs) and parvalbumin-expressing interneurons (PV-INs). On pyramidal cells, SOM-INs make distal dendritic synapses, while PV-INs make perisomatic synapses. We first discuss the properties of these interneurons and the circuits in which they are embedded to set the theoretical background of this thesis work. Then, we reviewed the diverse forms of inhibitory synaptic plasticity in the literature that mostly disregards the contribution of specific interneuron types. Lastly, through various means of triggering synaptic activity in brain slices, including NMDA application, electrical stimulation and optogenetic activation, in this study, we aim to experimentally compare and contrast the inducibility of plasticity of these two major types of GABAergic synapses on pyramidal cells. In our hands, NMDA activation potentiates inhibition mediated by SOM-INs (138.48% increase), while leaving PV-IN synapses unchanged, suggesting that NMDAR activation preferentially modifies distinct types of inhibitory synapses. Our evidence suggests that this plasticity involves postsynaptic changes. While on average, neither electrical nor optogenetic afferent stimulation induced plasticity in both synapses studied, further analysis of individual experiments suggest multiple forms of plasticity were induced. Of note, PV-IN synapses showed a greater predisposition to plasticity than those formed by SOM-INs (90.9% vs 60%). In both synapse types, long-term depression was most frequently observed but with apparently different expression mechanisms. Thus, through discussion of existing literature and experimental studies, we propose that GABAergic synapses are molecularly diverse and follow specific plasticity rules that is linked to the identity of the presynaptic interneuron.

KEY WORDS:

Inhibitory plasticity, NMDA, SOM-IN, PV-IN, 20 hz stimulation

ABBREVIATIONS:

AP: action potential

ACSF: Artificial cerebrospinal fluid.

IPSC: Inhibitory postsynaptic current.

EPSC: Excitatory postsynaptic current.

INs: Interneurons.

PV-INs: Parvalbumin expressing interneurons.

SOM-INs: Somatostatin expressing interneurons.

VIP-INs: Vasoactive peptide expressing interneurons.

NMDAR: N-Methyl-D-Aspartic acid receptor (Glutamatergic).

AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (glutamatergic)

FS: Fast spiking

PFC: Prefrontal cortex.

LTP: Long-term potentiation.

LTD: Long-term depression.

iLTP: Inhibitory long-term potentiation.

iLTD: inhibitory long-term depression.

SOM-synapse: inhibitory synapse mediated by SOM-IN.

PV-synapse: inhibitory synapse mediated by PV-IN.

VGCC: Voltage gated calcium channel.

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1.- INTRODUCTION:

For an efficient neuronal and cerebral development, the balance between excitatory (E) and inhibitory (I) activity plays a fundamental role. This E-I balance is dependent on coordinated changes in synaptic excitation and inhibition (Chiu et al., 2018, Kim et al., 2016, Sprekeler, 2017, Yang and Sun, 2018) and enables complex information processing associated to memory and behavior (Karnani et al., 2016). To study this relationship, different studies have focused on plasticity in excitation (Malenka and Bear, 2004) and more recently in inhibition (Chiu et al., 2018, Castillo et al., 2011).

In the brain, long-range communication is primarily mediated by excitatory neurons. In contrast, inhibitory neurons, commonly called interneurons, make local synapses. (Bittner et al., 2017). Interneurons play an important role in the maintenance of neuronal circuit function, and they are necessary for the establishment of long-term memory (Kim et al., 2016). However, it remains unclear how inhibition performs these fundamental operations. Inhibitory cells are less numerous than excitatory cells, comprising about 12% of all neurons in the neocortex (Feldmeyer et al., 2018). There are a lot of different types of interneurons which can be classified according to their firing properties, intrinsic membrane excitability, synaptic dynamics and morphology. Thus, it has been difficult to determine the contribution of specific types of interneurons in the regulation of pyramidal cell activity (Neske and Connors, 2016, Sprekeler, 2017).

The three main groups of interneurons in the cortex co-express either the calcium-binding protein Parvalbumin (PV-IN), the peptide transmitter Somatostatin (SOM-IN) or the serotonin 5HT3a receptor, the latter of which includes cells that express vasoactive intestinal peptide (VIP-IN) (Chiu et al., 2018, Feldmeyer et al., 2018, Rudy et al., 2011). Also, each of these groups can be subclassified by their morphology, the layer where their soma is located, and their intrinsic molecular biology. In this sense, 7 types of PV-INs and 6 types of SOM-INs have been described (Feldmeyer et al., 2018). Here, we investigated the regulation of inhibition mediated by PV-INs and SOM-INs, which are widely distributed in the cortex, and have their own morphological, physiological and connectivity characteristics (Kim et al., 2016). The PV-IN regulates the perisomatic region of pyramidal cells through synapses on its soma and proximal dendrites. It generates a fast and powerful inhibition, which regulates the

firing activity of pyramidal cells (Feldmeyer et al., 2018). In contrast, the SOM-IN synapses make synaptic inputs on the distal dendritic tree of pyramidal cells, and thus, they generate local inhibition near synaptic inputs, and regulate the evoked calcium (Ca^{+2}) signals, synaptic integration and dendritic excitability (Chiu et al., 2018, Kim et al., 2016). Regarding their interaction with other interneurons, PV-INs strongly inhibit each other, while SOM-INs inhibit other interneurons, but not other SOM-INs. The evidence suggests that both types of interneurons influence the neuronal circuit in different ways (Chiu et al., 2018).

GABAergic neurons are necessary for stability in neuronal dynamics. To do this, these cells must be adaptable, which may occur through homeostatic or Hebbian mechanisms. In this study, we discuss these forms of inhibitory plasticity. In the homeostatic framework, high activity in neuronal networks, usually indicative of a diminution of inhibition, may be counteracted by triggering a potentiation of inhibitory synapse (Vogels et al., 2011). Opposite to this, a low state of neuronal activity may indicate an excess of inhibition, which could be counteracted by triggering a depression in inhibitory synapses (Sprekeler, 2017). Both SOM and PV-INs show synaptic potentiation, but the underlying mechanisms and their impact on synaptic circuits may differ. For example, the activation of the NMDA-type glutamate receptor (NMDAR) can induce inhibitory long-term potentiation (iLTP) in synapses formed by SOM-INs, but not mediated by PV-IN (Chiu et al., 2018). Therefore, it was concluded that dendritic glutamatergic activity influences SOM-IN inhibition. On the other hand, inhibition mediated by PV-INs is strongly influenced by postsynaptic firing in pyramidal cells (Lourenco et al., 2014) which makes the postsynaptic cell itself capable of controlling synaptic inputs onto them.

To determine the mechanisms underlying plasticity of inhibition mediated by SOM-INs and PV-INs, we assessed changes in the inhibitory postsynaptic current (IPSC) evoked by optogenetic activation of either interneuron type in layer 2/3 pyramidal cells in PFC. Different approaches including bath application of NMDA, electrical and optogenetic stimulation were used to induce to induce endogenous glutamate release from excitatory inputs. Taking advantage of these interventions, we study glutamatergic contribution in the induction and expression of different forms of GABAergic plasticity.

2.- THEORETICAL FRAMEWORK:

2.1 Interneurons:

Inhibitory neurons play important roles in the development and maintenance of neuronal circuits. In the cortex, they configure nearly 20% of all cortical neurons, but their axons ramify extensively allowing them to control the cortical activity (Chiu et al., 2019, Rudy et al., 2011). They release GABA and it has a fundamental importance in modulating cortical function. GABAergic interneurons control the specific spatiotemporal aspects of the neuron activity, control the timing of pyramidal cell firing, synchronize network activity and cortical rhythms, and maintains the excitatory and inhibitory balance necessary for transfer information while preventing runaway excitation. There are different GABAergic interneurons according to their morphology, intrinsic membrane properties, connectivity, the efficacy and dynamic of input and output synapse and the expression of specific molecular markers. This diversity is critical to help in the variability functions of these neurons in the cortex, and in the form that they contribute to this (spatially and temporally) (Rudy et al., 2011).

2.1.2. Interneurons expressing Parvabumin:

These interneurons are characterized to have a fast-spiking firing pattern. There are two types of PV-IN: basket cells and chandelier cells. Basket cells typically have multipolar morphology and make synapses at the soma and proximal dendrite of target neurons. Chandelier cells or also known axo-axonic neurons (Tremblay et al., 2016), make synapse with the axon initial segment of pyramidal cells. The basket cell would be the dominant in this group. Another type of PV-IN is the “multipolar bursting cells” found mainly in upper layer 2. They have not been extensively studied (Tremblay et al., 2016, Rudy et al., 2011, Chiu et al., 2019).

Fast spiking (FS) cells fire high frequency trains of action potentials (AP) with a large and fast “after hyperpolarization”, and with little spike frequency adaptation (Chiu et al., 2019). When their potential exceeds the threshold, they fire abrupt episodes of non-adapting repetitive discharges. They have a low input resistance and fast time constant, contributing to

generating fast synaptic response. Because of that, they participate in quickly suppression of the output of target neurons, which is required in feedforward inhibition (Rudy et al., 2011).

In neocortex, PV-INs are the largest interneuron population and they have stereotypical fast and non-adapting firing patterns. The speed and precision of FS basket cell signaling are impressive: the delay between the action potential in a FS basket cell and the start of inhibitory postsynaptic current in pyramidal cell is short (average: 0.7 ms) (Tremblay et al., 2016). Due to these characteristics, they are considered the dominant inhibitory system in the neocortex, and in layer 4 they are the main interneuron target of thalamic input. This powerful inhibition by PV-IN contributes in the feedforward inhibition in cortical circuits, establishment and maintenance of fast cortical rhythms (gamma frequency) and regulation of critical experience-dependent plasticity (Rudy et al., 2011).

2.1.2. Interneurons expressing somatostatin:

SOM-INs target dendrites of pyramidal cells, making contacts onto shafts and spines. For this reason, SOM-INs restrict the temporal and spatial spread of both electrical and biochemical signals in pyramidal cells (Chiu et al., 2019). Of note, SOM-INs are activated when the network activity increases due to the facilitating excitatory inputs onto these interneurons from local pyramidal neurons. This connectivity pattern increases feedback inhibition mediated by SOM-INs when regional activity increases. SOM-INs are also powerfully excited by cholinergic activity (Rudy et al., 2011, Tremblay et al., 2016). Thus, dendritic inhibition generated by these interneurons can influence glutamatergic plasticity through regulation of Ca^{+2} activity which may be neuromodulated (Chiu et al., 2019).

By their morphology, SOM-INs in the neocortex are grouped into “Martinotti” and “non-Martinotti” cells. “Martinotti cells” have long and ascending axons that arborize in layer 1 spreading horizontally to neighboring columns and making synapses with the tuft dendrites of pyramidal cells. Their somas are principally in layer 5, but they can be found throughout layers 2 to 4 as well. In contrast, “non-Martinotti cells” do not branch in layer 1, therefore affecting the circuit in a different way SOM-INs fire in diverse patterns, including regular

adapting spikes (RSNP) and low-threshold spikes (LTS) (Tremblay et al., 2016, Rudy et al., 2011).

2.1.3. Interneurons expressing 5HT3aR:

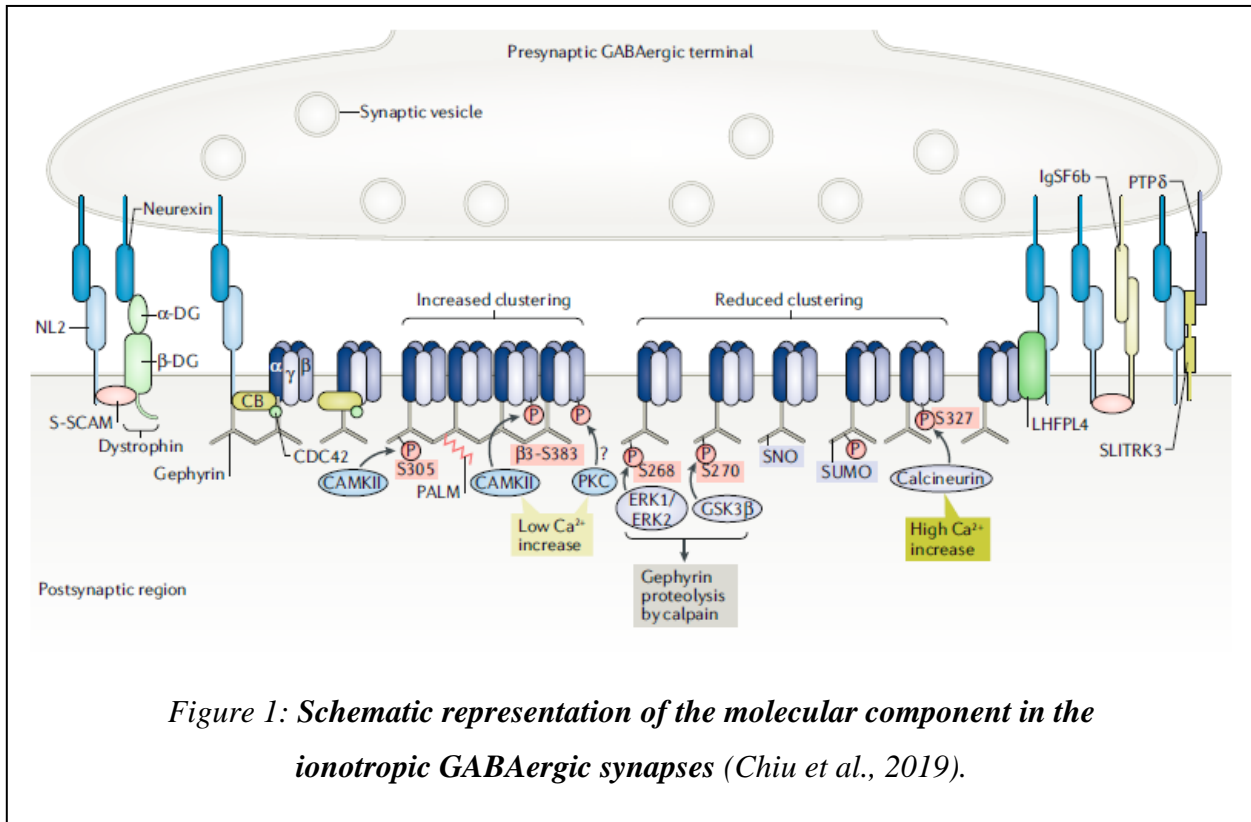
In somatosensory cortex (S1), they compromise nearly 30% of all GABAergic neurons and are the principal interneurons in superficial layers (1 to 3) configuring nearly 60% of all interneurons in these layers. In layer 1, almost all neurons are 5HT3aR-expressing. These interneurons have in common the expression of the 5HT3a receptor, and thus are activated by the serotonin neurotransmitter. Interestingly, they are also sensitive to cholinergic agonists (Tremblay et al., 2016, Rudy et al., 2011).

This group of interneurons is more heterogeneous than PV and SOM group, and thus is not completely characterized. The principal classification includes two types: cells that express vasoactive intestinal polypeptide (VIP) and cells do not express it. The VIP neurons are bipolar or bitufted neurons, with irregular spiking or fast adapting firing patterns. Also, they are particularly enriched in layers 2/3 (Rudy et al., 2011), but their dendrites extend into the superficial layer 1, reaching close to the pial surface. VIP neurons are better known for their disinhibition action because they target with other interneurons (Chiu et al., 2019). The non-VIP neurons represent nearly 60% of all 5HT3aR interneurons and include neurogliaform or multipolar neurons, cholecystokinin (CCK)-expressing interneurons, and other less clearly defined types (Tremblay et al., 2016), that inhibit pyramidal cell dendrites, exerting a powerful suppressive action on network activity (Chiu et al., 2019).

2.2 GABA synapse:

Inhibitory synapses require a variety of elements that help to support stable and yet dynamic inhibition. Fast inhibition needs the enrichment of type A GABA receptors (GABA_AR) at postsynaptic sites, that is achieved through interactions with postsynaptic scaffold proteins. GABA_AR is a ligand-gated ion channel whose activation allows chloride and bicarbonate ion flow. Structurally, it is a pentameric channel with a mixture of subunits that determine its functional properties and subcellular expression profiles.

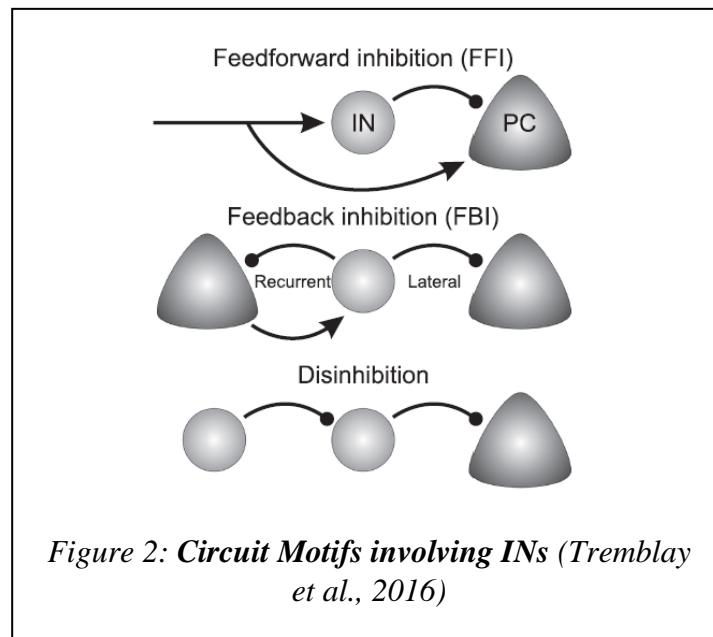
Some proteins that participate in ionotropic inhibitory synapse are: Gephyrin, collybistin, neuroligin 2/3 (NL2/3), dystrophin-glycoprotein complex (DGC), S-SCAM, LHFPL4. All of them participate in the establishment of inhibitory synapses and in the clustering process. Gephyrin is major component of scaffold for GABA_AR, interacting with the $\alpha 1$ - $\alpha 3$ subunit of the channel. For that reason, Gephyrin is important in structural stabilization of inhibitory synapse, but is not the only one in charge of it. Also, this interaction includes guanine nucleotide exchange factor collybistin (CB) forming the CB-gephyrin-GABA_AR complexes. Deletion of CB weakens inhibitory synapse. Also, other structures maintain the structure of GABAergic synapse including NL2, a postsynaptic adhesion protein that binds with: neurexin, a presynaptic adhesion protein; gephyrin, activating CB and inducing the recruitment of gephyrin and GABA_AR at postsynaptic sites; postsynaptic adhesion protein ST3 (also known as SLITRK3); and indirectly with another adhesion protein IgSF6b and with dystrophin-glycoprotein complex (DGC), both through the synaptic scaffolding molecule S-SCAM. DGC have three domains: extracellular (α -dystroglycan) that bind with presynaptic neuroxin; transmembrane (β -dystroglycan); and intracellular (dystrophin) that bind with S-SCAM. Recently a transmembrane accessory protein, LHFPL4, has gained importance for stabilization of GABA_AR inhibitory synapse, because it tightly binds GABA_AR and NL2 (Figure 1) (Chiu et al., 2019). In presynaptic surface other proteins are important to maintain stability of synapse and GABA release. Between them, at least five protein families: Munc13s, RIMs, piccolo/bassoon, α -liprins and ELKS. RIMs and ELKS are biochemically central elements because they bind to each other and to all other active zone proteins (Kaeser et al., 2009). Ongoing work continues to determine the specific molecular toolkit of distinct GABAergic synapses (Gibson et al., 2009, Panzanelli et al., 2011, Horn and Nicoll, 2018).



2.3 Inhibitory connectivity:

Local GABA release induces in the target cell a decrease of membrane potential known as inhibitory postsynaptic potential (IPSP), and along with this, changes in membrane resistance by the local conductance change (shunting inhibition). The influence in the postsynaptic cell depends on the location and strength of the GABAergic synapses. In case of pyramidal cell, the main excitatory neuron in cortex, there are several possibilities of this because their morphology (distal dendrites, proximal dendrites, soma or initial segment of axon). Generally speaking, perisomatic inhibition is most effective at counteracting neuronal spiking due to its action near the spike initiation zone, whereas dendritic inhibition acts like a gate for input integration in specific dendritic branches and regulates Ca^{+2} signals that may be important for synaptic plasticity (Fishell and Rudy, 2011, Koch et al., 1983, Liu, 2004).

Because of the above mentioned, the loci of proximal and distal inhibitory are crucial to determine if the dendritic signaling and action potentials will be temporally coupled or occur independently. The impact of the INs in the cortex, also depends on how it is embedded in the circuit. The main form to describe this interaction is feedforward inhibition (FFI), feedback inhibition (FBI) and disinhibition (Figure 2). These forms are present in all types of INs, however, specific subtypes could exhibit a more potent role in one circuit over another (Tremblay et al., 2016).



FFI happens when the excitatory input source on a principal neuron, also makes synapse with local inhibitory neuron generating disynaptic inhibition onto the neurons that receive the excitatory input. Conceptually this mechanism would participate in the regulation of the local activity triggered by incoming inputs, independently of the local network activity. In the majority of cases, FFI is mediated by PV-INs. The most studied excitatory input involved in this process is from thalamocortical neurons, where unilateral thalamocortical connections onto PV-IN are observed to be faster and 4-fold stronger than those onto pyramidal cells. As a result, few excitatory inputs are required to activate PV-INs, which in turn make strong GABAergic synapses onto pyramidal cells with a powerful disynaptic inhibition. Because FFI requires two synapses, it lags behind the monosynaptic excitation to the pyramidal cell. The

short delay is important in processes like sensory coding, setting a small window for excitatory activation (Tremblay et al., 2016, Simons and Carvell, 1989, Cruikshank et al., 2007, Alonso and Swadlow, 2005).

In the second inhibitory interaction, FBI occurs when the source of excitation is generated in the local region, from the same pyramidal cell population which receives the inhibition. For this reason, the principal function of FBI is thought to be reducing or preventing further discharges of excitatory cells. The interneurons involved in this interaction show particular recruitment patterns and provide different functional features to the local pyramidal cell, and specifically they participate in the excitatory-inhibitory balance of the local neuronal population. In contrast to FFI that depends on extracortical inputs, FBI depends on intracortical activity. Because of the divergence of interneuron connectivity, the inhibitory cell will not inhibit a group of pyramidal cells in the circuit, generating lateral inhibition (Tremblay et al., 2016, Adesnik et al., 2012, Gonchar and Burkhalter, 2003, Yang et al., 2013).

Finally, disinhibition happens when one GABAergic interneuron primarily forms synapses onto other GABAergic interneurons. In this context, the main function of the interneuron to inhibit other GABAergic interneurons more potently than pyramidal cells. Although all types of interneurons could participate with some grades of disinhibition, the VIP-INs have been strongly involved in this (Caputi et al., 2009, Lee et al., 2013, Fu et al., 2014, Zhang et al., 2014). Interestingly, in cortical layer 4, the connection probability and synaptic strength of SOM-INs are much larger on PV-INs than on pyramidal neurons. For this reason, the activity of SOM-IN in layer 4 produces disinhibition in pyramidal cells by inhibition of PV-INs (Tremblay et al., 2016, Kisvarday et al., 1993, Xu et al., 2013, Beierlein et al., 2003).

2.4 Inhibitory plasticity:

Synaptic plasticity can be short-lasting and long-term. Plasticity of excitatory synapses has been more extensively studied than inhibitory synapses (Chiu et al., 2019) because of two principal reasons: 1) the difficulty to study inhibitory synapses due to the requirement to isolate specific types (Castillo et al., 2011); and 2) the compounded effect on inhibition by the excitatory and inhibitory synapses onto the interneurons (Kullmann et al., 2012). In this way, inhibitory plasticity may be mediated by changes in the inhibitory connections per se, as well as by plasticity in the excitatory inputs to inhibitory neurons (Chistiakova et al., 2019).

GABAergic plasticity can regulate excitability in the neuronal circuit, contribute to learning and memory and participate in neuronal refinement. In general, the plasticity can be induced by changes in the presynaptic GABA release or change in the number, or responsiveness of postsynaptic GABA_AR receptors (Castillo et al., 2011). The signaling cascades for inducing these changes involve protein kinase A and C (PKA and PKC), Ca⁺²/calmodulin-dependent kinase II (CamKII) and tyrosine kinases (Kullmann et al., 2012).

2.4.1 Molecular mechanisms:

Diverse forms of plasticity require a postsynaptic Ca⁺² rise, and it has been reported in excitatory and inhibitory synapses (Bannon et al., 2020). It is believed that the specific magnitude and dynamics of Ca⁺² rise determines whether long-term depression (LTD) or potentiation (LTP) is induced. This has been determined through manipulation of the increase of Ca⁺² in the interneurons with different calcium buffers, in such a way that the reduction of Ca⁺² rise generated in “LTP-protocol” can induce LTD instead (Bienenstock et al., 1982, Lisman, 1989, Lisman, 2001).

In general, this postsynaptic Ca⁺² signal is generated by ligand-gated ion channels (NMDAR, calcium permeable (CP)-AMPA and CP-AChR, ligand-gated metabotropic receptors (mGluRs), voltage-gated calcium channels (VGCC), and indirectly by depolarization such as in back-propagating action potentials (bAPs). Also some of those processes allow the release of intracellular sources of Ca⁺² from endoplasmic reticulum (Bannon et al., 2020).

NMDAR is ubiquitous in dendritic spines of pyramidal cells, and for that reason, participates in both LTP and LTD of excitatory synapses. On the other hand, in interneurons, the expression and contribution to Ca^{+2} signals is variable depending on the interneuron type and their connections and positions. It has been reported that this variability is higher in SOM-INs, with respect to PV-INs (Bannon et al., 2020). At the inhibitory synapse, activation of postsynaptic NMDARs in layer 2/3 pyramidal neurons of the prefrontal cortex induces a strengthening of dendritic inhibition from SOM-INs (Chiu et al., 2018). This involves the insertion of additional GABA_ARs into the synaptic membrane and requires CAMKII activity. Presynaptic NMDARs in GABAergic terminals can also induce LTD (Lien et al., 2006, Liu et al., 2007) or LTP (Liu and Lachamp, 2006, Lachamp et al., 2009) through the increase of Ca^{+2} in the terminal. In both cases, the glutamate from neighboring excitatory cells activate NMDARs in presynaptic cells. The LTP occurs specifically in stellate cells which release more GABA when the Ca^{+2} influx in the terminal activates cAMP/PKA. The specific mechanisms for inducing LTD or LTP via presynaptic NMDA, is not clear yet (Castillo et al., 2011).

Similar to NMDARs, certain AMPARs can be calcium permeable (without the GluR2 subunit) (Jonas and Burnashev, 1995, Rozov and Burnashev, 1999). In the interneuron, the proportion between CP vs CI-AMPARs differs with respect to cell type and region. In general, synapses with more NMDARs contain less CP-AMPARs (and more CI-AMPARs), and vice versa. The contribution to the Ca^{+2} influx through AMPAR and NMDAR depends on the membrane potential: hyperpolarization favors influx through AMPARs, while depolarization favors NMDARs, when magnesium block is released (Bannon et al., 2020, Sambandan et al., 2010, Lei and McBain, 2002).

A group of interneurons in CA1 stratum oriens of hippocampus express calcium-permeable acetylcholine receptors (CP-AChRs), these interneurons are somatostatin and neuropeptide Y (NPY) expressing. The Ca^{+2} influx via CP-AChR would be necessary for the induction of NMDA-independent “anti-Hebbian” LTP (Jia et al., 2010, Griguoli et al., 2013).

Respect to mGluRs, depending on type, they contribute in different forms of Ca^{+2} rise. The fast Ca^{+2} signals were mediated by mGluR1 α leading to activation of transient receptor potential (TRP) channels and release from internal stores; while slow Ca^{+2} signals were

mediated by mGluR5 (Topolnik et al., 2005, Topolnik et al., 2006). These receptors can be recruited by high frequency or theta-burst stimulation (TBS). They can contribute to plasticity of interneurons in different ways, e.g., LTP in stratum oriens neurons (mGluR1 α), LTP in layer 2/3 interneurons from visual cortex, LTD in FS interneurons from somatosensory cortex, and timing-dependent LTP or LTD in PV-INs from visual and somatosensory cortices (Sarihi et al., 2008, Lu et al., 2007, Huang et al., 2013). mGluR could play in the switch from LTD to LTP, because the activation of mGluR combined with other calcium sources can induce the Ca⁺² rise necessary to induce LTP, but when this receptor is not activated, the Ca⁺² signals generated by the other sources may not be enough for LTP induction, but this may be sufficient to induce LTD (Bannon et al., 2020, Hainmueller et al., 2014).

Another source of Ca⁺² is VGCCs, being a nonsynaptic mechanism that involve not only the dendrite contacted by active synapse, but also can involve dendritic branches and even the whole dendritic tree (Bannon et al., 2020). VGCCs are expressed in the dendrites of pyramidal cells and interneurons, and they are activated thanks to depolarization such as that during the backpropagation of action potentials. Dendritic Ca⁺² signals produced by backpropagation have been reported in all interneurons reported and require sodium channels. The Ca⁺² signals during bAPs are mediated by VGCCs and amplified by Ca⁺² release from internal sources. In addition, bAPs can enhance Ca⁺² signals mediated by NMDARs in SOM-INs and PV-INs from layer 2/3 of visual cortex, as well as Ca⁺² rise mediated by mGluRs in dendrites of perisomatic inhibitory neurons in dentate gyrus. Interestingly, bAPs have also been observed to reduce Ca⁺² signals mediated by CP-AMPARs (Bannon et al., 2020, Martina et al., 2000, Sancho and Bloodgood, 2018, Hainmueller et al., 2014) and in inhibitory synapses onto pyramidal cells, can participate to decrease presynaptic GABA release transiently. In this case, it is suggested that glutamate, packaged in dendritic vesicles by vGLUT3, would be released in response to the bAP in the pyramidal cell and retrogradely acts on presynaptic mGluRs (Kullmann et al., 2012).

The increase in Ca⁺² can trigger many signaling transduction processes that are involved in different forms of plasticity. Below, we discuss a few mechanisms that are thought to underlie presynaptic forms of inhibitory synaptic plasticity that are characterized by a change in GABA

release, as well as the postsynaptic forms of plasticity that involves changes in GABAR number and/or function (Castillo et al., 2011, Chiu et al., 2019).

- Nitric oxide (NO):

This molecule is retrogradely released from postsynaptic surfaces generating a presynaptic change in the inhibitory synapse. Somatic depolarization of cortical layer 5 was sufficient to trigger calcium-dependent mobilization of NO, which diffuses to inhibitory axon terminals to increase GABA release from PV-INs, but not from SOM-INs (Lourenco et al., 2014). This is because the terminals of PV-INs express NO-sensitive guanylyl cyclase (NO-cGMP) (Vruwink et al., 2001). This form of LTP is triggered by high frequency stimulation of glutamatergic fibers and this induction requires NMDAR activation, postsynaptic Ca^{+2} rise, and NO-cGMP. This circuit has been implicated in drug addiction (Castillo et al., 2011).

- Brain-derived neurotrophic factor (BDNF):

BDNF can be secreted from either axon terminals or dendritic compartments in response to excitation and can modulate both excitatory and inhibitory transmission. When BDNF is released from postsynaptic dendrites, it targets presynaptic tyrosine receptor kinase B (TRKB), which is preferentially expressed at axonal terminals of PV-INs to modulate calcium-dependent GABA release. This interaction increases GABA release (Castillo et al., 2011, Chiu et al., 2019). To postsynaptically release BDNF, an increase of Ca^{+2} in the dendritic compartment is required, and the source may be NMDARs, L-type VGCCs or Ca^{+2} release from internal stores, the latter being the most likely. Other than increasing GABA release, BDNF has been shown to increase the number of GABAergic terminals and to regulate the expression of the chloride transporter KCC2 which determines the mature intracellular chloride concentration. iLTP mediated by BDNF occurs in a restricted time window when GABAergic circuits are maturing. The chronic application of BDNF to cultured neurons increases both the size and the number of GABAergic terminals (Castillo et al., 2011).

- Endocannabinoids (eCBs):

Endocannabinoid is a retrograde messenger involved in inhibitory plasticity, but different to those mentioned above, it induces a decrease of GABA release from presynaptic interneurons. Postsynaptic depolarization or activation of metabotropic receptors induces the synthesis of

eCBs and they diffuse across the membrane to presynaptic sites to activate type 1 cannabinoid receptors (CB1Rs). CB1Rs then modulate terminal Ca^{+2} influx and reduce neurotransmitter release transiently or persistently (Castillo et al., 2011, Chiu et al., 2019). (Castillo et al., 2011, Chiu et al., 2019). At GABAergic synapses, a train of action potentials triggers a short-lasting inhibitory plasticity, known as depolarization-induced suppression of inhibition (DSI) (Chiu et al., 2019).

Additionally, it has been reported that glutamatergic activity also participates in LTD mediated by eCBs. In the hippocampus, glutamate from Schaffer collaterals can activate postsynaptic group 1 metabotropic receptors (mGlu1) and induce eCB production and consequently the reduction of GABA release from presynaptic terminals (Chevalyere et al., 2006, Garkun and Maffei, 2014, Diana and Marty, 2004). Also, a similar process has been evidenced in amygdala, dorsal striatum, brainstem, and visual cortex (Castillo et al., 2011). The synthesis of eCBs can also be stimulated by phospholipase C activation by muscarinic M1/M3 receptors (Kullmann et al., 2012).

Other factors that regulate this process are depolarization and firing from the interneuron, instilling cooperativity in eCB-iLTD between presynaptic and postsynaptic activity. CB1Rs are metabotropic Gi/o-coupled receptors whose activation results in a decrease in the adenylyl cyclase-PKA transduction cascade, subsequently involving the calcium-sensitive phosphatase, calcineurin. Of note, the type 2 dopamine receptor (D2R) also can reduce PKA activity (Chiu et al., 2010, Pan et al., 2008). In PFC and VTA, D2Rs and CB1Rs work together to suppresses GABAergic transmission. The LTD generated by CB1R activation enhances the influence of glutamatergic inputs on action potential generation and can shift the excitatory/inhibitory balance toward excitation favoring signal propagation within and across neural networks (Castillo et al., 2011).

- GABA neurotransmitter and GABAbR:

In addition to ionotropic GABA_ARs, there exists a metabotropic b-type GABA receptor (GABAbR). GABAbR is Gi/o protein-coupled receptor that has been found in many neuron types including excitatory and inhibitory cells and it can be present on both postsynaptic dendrites or soma and presynaptic terminals (Tabata and Kano, 2010, Kantamneni, 2015). Unlike GABA_ARs, the GABAbR mediates slow and lasting inhibitory neurotransmission in

the central nervous system. GABA_BR is a heteromeric G protein-coupled receptor consisting of GABA_B1 subunit that have the ligand-binding domain, and GABA_B2 subunit that interacts with G-proteins to regulate adenylate cyclase, inwardly-rectifying potassium channel (GIRK channel) and Ca⁺² channels. Different from most G protein-coupled receptors that are phosphorylated and endocytosed from the cell surface when exposed to its agonist, cell surface GABA_BR levels were not significantly altered after being stimulated in cultured cortical and hippocampal neurons. Instead, GABA_BR is regulated by glutamate in such a way that activation of NMDARs and AMPARs is required for its down-regulation, and this effect is enhanced by the activation of mGluRs (Kantamneni, 2015).

In postsynaptic soma and/or dendrites, GABA_BR activation leads to opening of G protein-coupled inwardly rectifying K⁺ (GIRK) channels. Intracellular rise of K⁺ counteracts spontaneous or synaptically depolarization impeding the action potential (Kantamneni, 2015, Tabata and Kano, 2010, Chiu et al., 2019). In the presynaptic terminal, GABA_BR leads to inhibition of VGCCs. The reduced Ca⁺² influx decreases neurotransmitter release attenuating synaptic efficacy (Kantamneni, 2015, Tabata and Kano, 2010). These receptors also exhibit plasticity but the mechanisms and physiological impact are not well understood. Alterations of these receptors have been linked to seizure activity, but with an unclear signaling cascade (Chiu et al., 2019).

Surprisingly, GABA_BR is also widely distributed in excitatory synapses, and it has been shown that it can modulate glutamatergic receptor function (e.g., NMDAR, AMPAR and mGluR) directly and indirectly (Chalifoux and Carter, 2010, Gandal et al., 2012, Terunuma et al., 2014). The Ca⁺² signals mediated by NMDARs are inhibited by GABA_BR activation in two complementary processes; the first thanks to the modulation of potassium channels, resulting in hyperpolarization of postsynaptic cells that improves the magnesium blockade of NMDAR. The second is independent of potassium channels, VGCCs and internal stores, and instead is induced by the signaling cascade generated by the activation of the G_i protein. Its activation inhibits adenylate cyclase, decreasing cAMP, which in turn reduces PKA activity. In this case, the Ca⁺² influx via NMDARs is decreased by reduced PKA activity (Kantamneni, 2015).

One of the sites where this channel is distributed is in the cerebellum and specifically in Purkinje cells (Fernandez-Alacid et al., 2009). Here, the majority of the GABA_ARs resides in dendritic spines innervated by excitatory (glutamatergic) but not inhibitory (GABAergic) presynaptic neurons and when ligand binds to GABA_ARs, an enhancement of neuronal responses mediated by mGluRs is generated. Variations in Ca⁺² concentration in the synaptic space have been reported to induce a conformational change in the GABA_AR that modulates its binding property with GABA. Thus, the extent of Ca⁺² present alters the affinity of the receptor with the neurotransmitter (Tabata and Kano, 2010).

At the synapse made by pyramidal cells on bitufted interneurons in rat neocortex, a decrease of glutamate release by presynaptic GABA_AR activation has been reported. It is thought that this GABA could be from dendrites of postsynaptic interneurons, and in this case, GABA acts as a retrograde messenger (Kullmann et al., 2012).

3.- RESEARCH PROBLEM

3.1 Research Question:

How are GABAergic synapses from different interneurons regulated?

3.2 Hypothesis:

Repetitive afferent stimulation will trigger different forms of inhibitory plasticity depending on the presynaptic GABAergic interneuron type due to the specific molecular synaptic composition present.

4.- OBJECTIVES:

4.1 General Objective:

Study how inhibition mediated by somatostatin-positive GABAergic interneurons (SOM-INs) and by GABAergic interneurons positive for parvalbumin (PV-INs) is regulated by repetitive afferent stimulation.

4.2 Specific Objectives:

- To determine whether an increase in exogenous NMDA preferably modifies dendritic inhibition mediated by SOM-IN, relative to PV-IN.
- To determine if the repetitive activation of glutamatergic afferences differentially modifies the dendritic inhibition mediated by SOM-IN, with respect to PV-IN
- To determine if repetitive activation of thalamic glutamatergic afferences modifies the dendritic inhibition mediated by SOM-IN.

5.- METHODOLOGY:

5.1 Animal welfare:

All the experiments and animal handlings were done according to the ethical protocol established by “National Institutes of Health” (USA) and approved by the ethics committee of the Faculty of Sciences of “Universidad de Valparaíso” (Chile).

5.2 Experimentation animals:

The animals used in this study were mice with specific interneurons Cre-driver lines: Som-cre +/+ and PV +/+ mice at postnatal day (P) 20 to 30 for injecting it, and P50 to P60 for use in electrophysiological experiments. Males and Females were used without distinction. Mice were housed in the Faculty of Sciences of “Universidad de Valparaíso ” on alternating 12-hour light-dark cycle and provided with food and water ad libitum.

5.3 Experimentation protocols:

5.3.1 Injection animal:

Virus injections were made for the expression of Chrimson channels (ChrimsonR) and Channelrhodopsin-2 (ChR2) in PFC and medial dorsal thalamus (MD thalamus), depending on the specific experiment. PFC was injected by conditional to -Cre virus, in such a way that the optogenetic receptor was selectively expressed in SOM-INs or PV-INs, depending on the mice. MD thalamus was injected by non-conditional virus, thus all cells in the place expressed the non-conditional optogenetic receptors. In experiments with exogenous NMDA and electrical stimulation, the mice were injected only in PFC; in experiment thalamic stimulation, the mice were injected in PFC and MD thalamus. The viruses used were rAAV5-Ef1a-DIO-ChR2-EYFP (conditional virus) and rAAV5-syn-ChR2-EYFP (non-conditional virus) for ChR2 expression; and rAAV5-hsyn-Flex-CrimsonR-tdT (conditional virus) for ChrimsonR expression (UNC Vector Core, North Carolina, EEUU).

In each injection was used 1 microliter of the virus. The injections were done between P20 and P30 in SOM-cre and PV-cre mice. In experiments that required PFC and MD thalamus injections, both were done during the same surgical procedure.

5.3.2 Slice preparation:

Then 2 to 4 weeks after virus injection, the brains were extracted and slices in PFC zone of 300 um thick were done, preparing electrophysiological experiments. For this, two external solutions were used: Cutting solution for brain extraction and make slices (in mM: 25 NaHCO₃; 1.25 NaH₂PO₄; 15 Glucose; 2.5 KCl; 7 MgCl₂; 0.5 CaCl₂; 110 choline chloride; 11.6 Ascorbate; 3.1 Pyruvate; bubbled with 95% O₂ and 5% CO₂); and artificial cerebrospinal fluid (ACSF) for maintain survival of slices during the experimental protocols (in mM: 127 NaCl; 25 NaHPO₃; 1.25 NaH₂PO₄; 15 Glucose; 2.5 KCl; 1 MgCl₂; 2 CaCl₂; bubbled with 95% O₂ and 5% CO₂).

5.3.3 Electrophysiological experiments:

Patch-clamp experiments in whole-cell mode were done in pyramidal cell of layer 2/3 in PFC. Inhibitory currents were measured in voltage-clamp modality, setting the voltage in 0 mV. The internal solution used was Cesium Gluconate (in mM: 125 gluconic acid; 275 CsOH; 10 HEPES; 4 MgCl₂; 4 Na₂ATP; 0.4 NaGTP; 10 Na₂CrePO₄; and in uM: 100 EGTA-CsOH).

To evoke the optogenetic response was used red light stimulation (wavelength: 595 nm) for ChrimsonR activation; and blue light stimulation (wavelength: 470 nm) for ChR2 activation. The light used depended on the specific experiment, which will be detailed in the next session.

5.4 Experimental design and data acquisition:

5.4.1 Effect of exogenous NMDA:

1. ChR2 –cre conditional virus was injected in PFC of both mice: SOM-cre +/+ and PV-cre +/+.
2. Voltage-clamp in pyramidal cells of layer 2/3 PFC with clamp in 0 mV for IPSC measurement. Blue light stimulation was used to induce IPSC mediated by SOM-IN or PV-IN depending on the mice.
3. Recordings of paired pulse stimulation were done. 2 pulses of 1 millisecond blue light and separated to 100 milliseconds, each 10 seconds. This for 10 minutes of duration. The first pulse was used for calculating basal IPSC, and the paired pulse ratio (PPR) for analysis of plasticity.
4. Then 10 minutes of basal activity, NMDA (20 uM) was added in the bath for 2 minutes. During this time and after 20 minutes, it continued to record with the same paired pulse stimulation.
5. Baseline activity (IPSC basal and PPR basal) was compared with the average of the last 5 minutes of experimentatio

5.4.2 Effect of electrical stimulation at 20 hz

In the previous experiment, the change in the inhibitory synapse in response to exogenous NMDA was studied. To induce endogenous glutamate, first we used electrical stimulation. How the principal inputs in PFC are from thalamus in layer 4, we put the stimulation electrode in this layer. The stimulation used was at 20 hz which corresponds to the thalamic trigger frequency in vivo (Steriade et al., 1991, Leresche et al., 2012).

1. Chrimson-cre conditional virus was injected in PFC of both mice: SOM-cre +/+ and PV-cre +/+.

2. Before patch pyramidal cell, stimulation electrode was put in layer 4 of PFC, in the direction of the pyramidal cell that would be registered.
3. Voltage-clamp in pyramidal cells of layer 2/3 PFC with clamp in 0 mV for IPSC measurement. Red light stimulation was used to induce IPSC mediated by SOM-IN or PV-IN depending on the mice.
4. Recordings of paired pulse stimulation were done. 2 pulses of 1 millisecond red light and separated to 100 milliseconds, each 10 seconds. This for 10 minutes of duration. The first pulse was used for calculation of basal IPSC, and the paired pulse ratio (PPR) for analysis of plasticity.
5. Then 10 minutes of basal activity, we used electrical stimulation: 20 pulse at 20 hz each 5 seconds twice (20x@20hz each 5 sec x2). After protocol, it continued to record with initial paired pulse stimulation (with red light).
6. Baseline activity (basal IPSC and basal PPR) was compared with the average of the last 5 minutes of experimentation.

5.4.3 Effect of optogenetic stimulation at 20 hz:

In the first experiment, we studied the plasticity generated by the application of the exogenous NMDA. In the second, theoretically the electrical stimulation induces glutamatergic release from thalamic inputs, but also can activate all the cells near the stimulation, inducing plasticity from different sources. For that reasons, we wanted to contrast these results and confirm if now the endogenous glutamate could induce similar effects. The glutamate release was from thalamic inputs and induced by optogenetic stimulation. This stimulation was at 20 hz which corresponds to the thalamic trigger frequency in vivo (Steriade et al., 1991, Leresche et al., 2012). Also, Chiu et al. using this protocol found that with this frequency inhibitory LTP is generated an inhibitory synapse mediated by SOM-IN. However, this protocol was not tested in the synapse mediated by PV-IN (Chiu et al., 2018).

Conditional ChrimsonR was expressed in SOM-IN to evoke IPSC by red light, while unconditional ChR2 was expressed in thalamic neurons to evoke glutamate release in PFC by

blue light. Previously we demonstrated that both channels work and activate with different colors of light. The ChrimsonR was preferably activated by red light, but also by blue light.

1. Chrimson-cre conditional virus was injected in PFC and ChR2 unconditional virus was injected in ipsilateral MD thalamus. Both injections in SOM-cre mice.
2. Voltage-clamp in pyramidal cells of layer 2/3 PFC. First, with red light (Conditional Chrimson activation), the reversal potential was determined. The cell was fixed in this potential and was stimulated by blue light. This to ensure that thalamic inputs have functional unconditional ChR2. In this situation the response with blue light corresponds to EPSC mediated by glutamate from thalamic neurons. After that and in the rest of the experiment, the pyramidal cells were fixed at 0 mV for measurement IPSC.
3. Recordings of paired pulse stimulation were done. 2 pulses of 1 millisecond red light and separated to 100 milliseconds, each 10 seconds. This for 10 minutes of duration. The first pulse was used for calculation of basal IPSC, and the paired pulse ratio (PPR) for analysis of plasticity.
4. Then 10 minutes of basal activity, we used blue light stimulation: 20 pulse at 20 hz each 5 seconds twice (20x@20hz each 5 sec x2). After protocol, it continued to record with initial paired pulse stimulation (with blue light).
5. Baseline activity (basal IPSC and basal PPR) was compared with the average of the last 5 minutes of experimentation.
6. This experiment was replied with MCPG at 10uM in the bath, to determine the influence of glutamatergic metabotropic receptors (mGluR). MCPG can block group I (mGluR1 and mGluR5) and group II receptors (mGluR2 and mGluR3)

5.5 Statistic analysis:

The IPSCs were analyzed through “Igor”. The amplitude of IPSCs was calculated like the response generated by the first optogenetic pulse. The “basal activity” corresponds to the 5 firsts minutes; and “final activity” like the final 5 minutes of experimentation.

Then the dates about IPSCs were put in “GraphPad Prism 8.0”, to make the normalization, graphs and statistical analyses. Wilcoxon Test was used to compare paired conditions with changes in the same cell: Basal vs Final IPSC in average, Basal vs Final PPR in average, and Basal vs Final IPSC in each cell to determine if the protocol induced LTP, LTD or nothing. A statistically significant difference was considered with $p < 0.05$.

Mann-Whitney test was used for comparing unpaired conditions in different cells: Final IPSC in one group vs other group (e.g. response in SOM vs response in PV). A statistically significant difference was considered with $p < 0.05$.

Different graphs were made: graph with change of IPSC over time for see the effect of stimulation in time, PPR change in each experiment, graph with change ratio of IPSC (Amplitude final / basal) and difference of PPR in each experiment, and graph with the percentage of cell in each experiment that presented plasticity.

6.- RESULTS:

6.1 Effect of exogenous NMDA:

To evaluate the effect of exogenous NMDA in the GABAergic synapses, 11 experiments were performed in SOM-cre mice, and 6 experiments in PV-cre mice (all injected previously with ChR2 virus in PFC). The final SOM-IPSC showed on average to 138.48% increase in the amplitude respect the basal with a statistically significant difference ($p < 0.05$) (Figure 3.A y 3.B), however the PV-IPSCs on average did not show a change in amplitude and without statistically significant difference ($p = 0.8434$) (Figure 3.B)

When we analyzed the PPR in those experiments, neither of both experiments showed a change with respect to their baseline PPR (Figure 3.D y 3.E). In the SOM-IN experiment, the mean baseline PPR was 0.6971 and final was 0.6818 ($n = 10$), while in the PV-IN experiment, the mean baseline PPR was 0.7335 and final was 0.6732 ($n = 4$).

In general, in SOM-IN experiments, 81.82% ($n = 9$) of cells showed iLTP ($p < 0.05$ before vs after NMDA), and 18.18% ($n = 2$) did not have a statistical change. With respect to the PV-IN experiments, 50% ($n = 3$) of cells showed iLTP, 33.33% ($n = 2$) had iLTD, and 16.67% ($n = 1$) did not have plasticity (Figure 3.F).

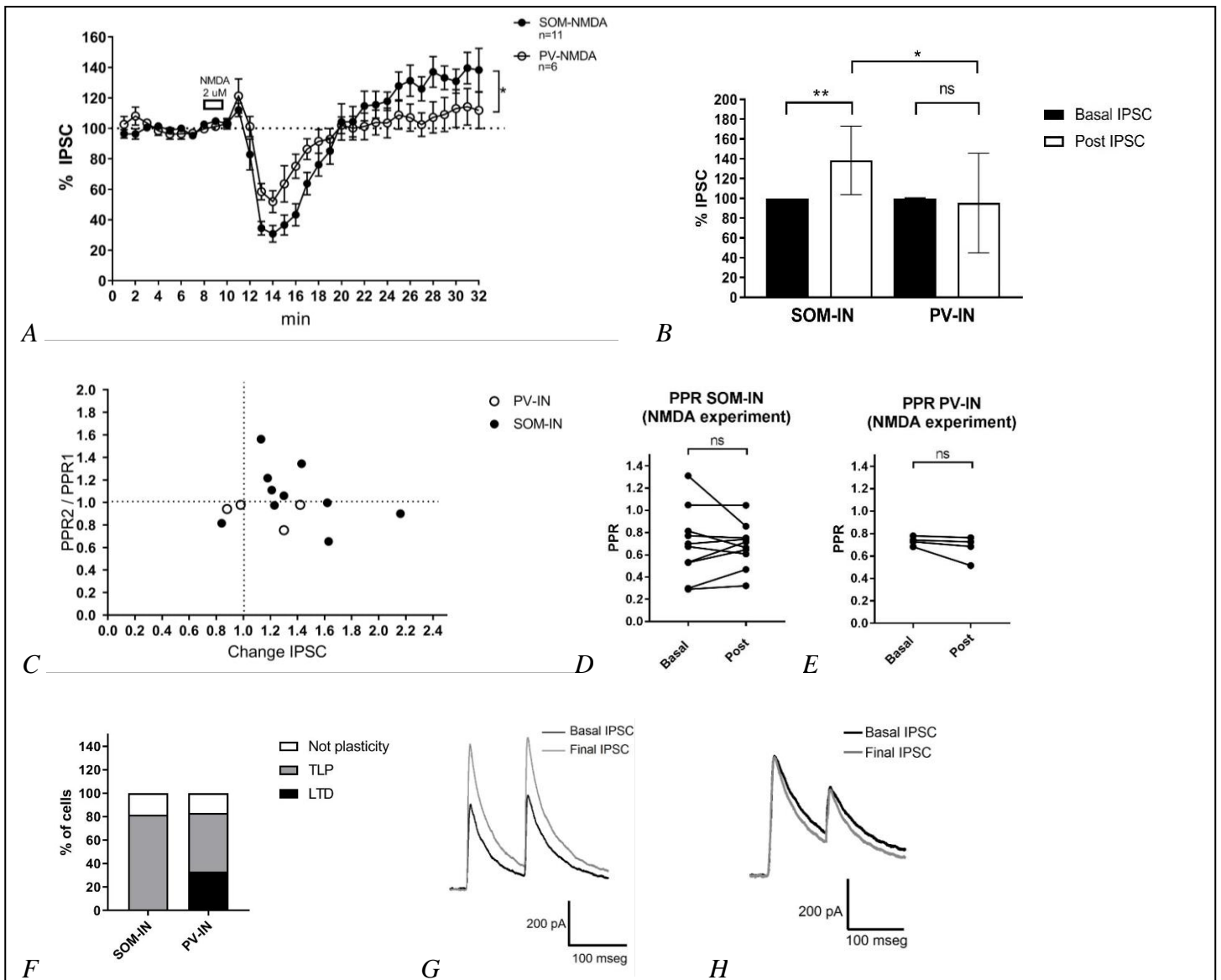


Figure 3. Effect of NMDA in IPSC mediated by GABAergic synapse. A: Time course of IPSCs mediated by SOM-IN (black circles) and by PV-IN (white circles). The white rectangle represents the time (2 minutes) of 20 μ M NMDA in bath. B: Comparison of basal (in black) and final (in white) IPSC in SOM and PV experiments. Also, the statistic different between them. C: Relation between change IPSC (Final IPSC / Basal IPSC) in X axis and Δ PPR (math difference between final PPR and basal PPR) in Y axis in each experiment (Black circle in SOM-IN and White circle in PV-IN). D: PPR change in each SOM experiment. E: PPR change in each PV experiment. F: Percent of cell in each experiment with LTD (black color), LTP (gray color) and without plasticity (white color). G: IPSC example in SOM-synapse. H: IPSC example in PV-synapse. (n.s.= not significant, * $p = 0.0370$, ** $p = 0.0029$).

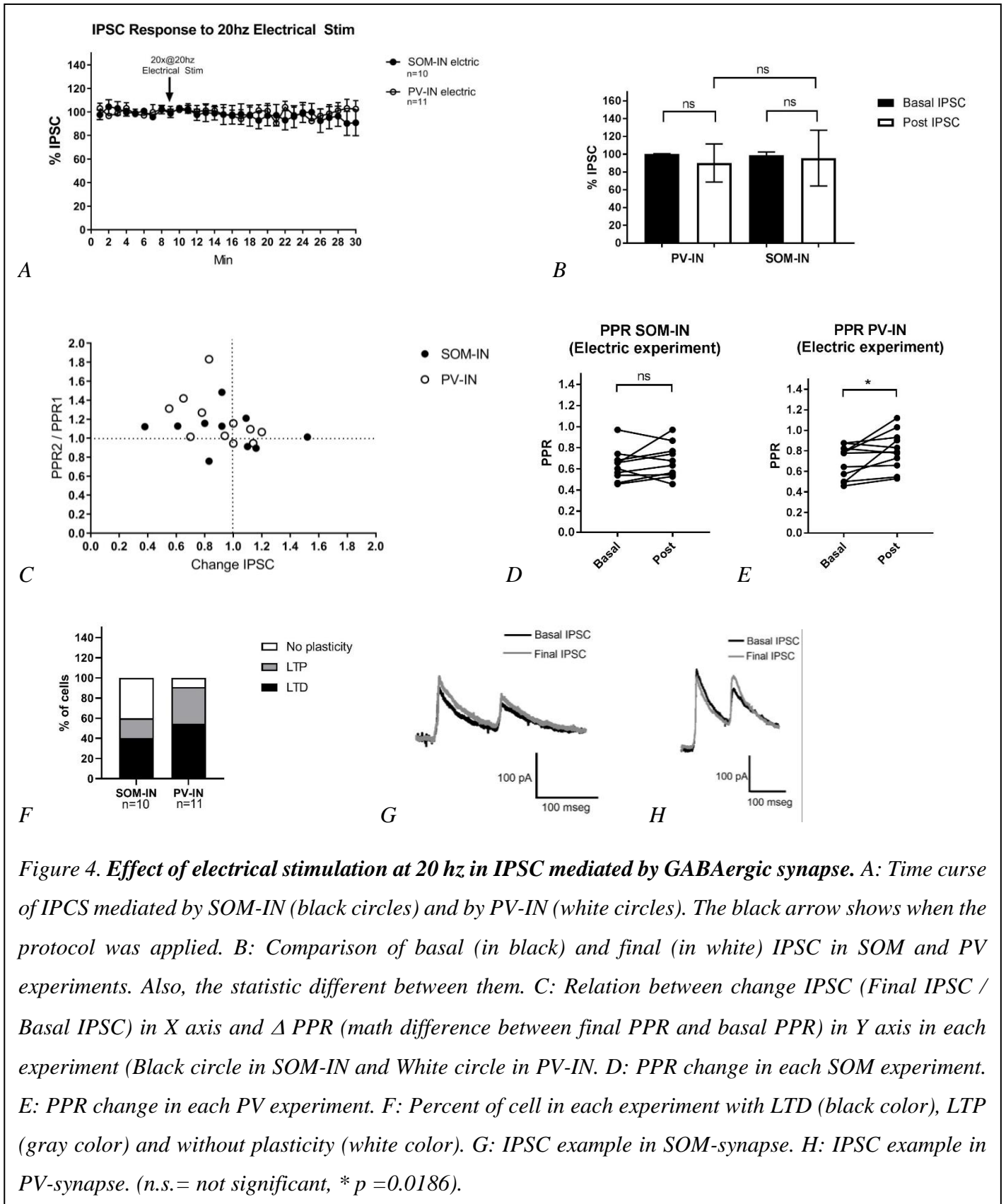
6.2 Effect of electrical stimulation at 20 hz

6.2.1 General

In the electrical stimulation protocol, we evaluated 10 cells of SOM-cre mice and 11 cells of PV-cre mice injected previously with Chrimson conditional to cre virus in PFC. On average, 20 hz electrical stimulation, for both SOM-IPSCs and PV-IPSCs did not show a statistically significant difference with respect to baseline ($p=0.5566$ and $p=0.1602$, respectively). Also, when we compared the final IPSC each other, and we did not find a statistical difference ($p=0.9313$) (Figure 4.A y 4.B). In the PPR analyses, the mean baseline in the SOM experiment ($n=10$) was 0.6334 and the mean final was 0.6761, without statistically significant difference between them ($p=0.4313$) (Figure 4.D). Surprisingly in the PV experiment ($n=11$), the mean baseline PPR was 0.6911, and the final showed a slight increase with a value of 0.8046 and also a statistically significant difference with basal PPR ($p<0.05$) (Figure 4.E).

The breakdown of SOM experiments is as follows: 20% of cells ($n=2$) had iLTP, 40% ($n=4$) had iLTD, and 40% ($n=4$) did not show a change in the amplitude of IPSCs. For PV experiments, the majority of cells presented a change: 36.4% ($n=4$) had iLTP, 54.5% ($n=6$) had iLTD and 9.1% ($n=1$) did not have plasticity (Figure 4.F).

Because of this variability, we considered it necessary to group the experiments by plasticity and study the different forms of plasticity in each group of cells to unravel expression mechanisms. We presented this in the next section.



6.2.2 SOM Experiments.

In the specific analysis of SOM experiments, the cells with iLTD had a mean decrease to 65.5%, and the cells with LTP a mean increase to 131%, but when we compared statistically the difference between the 3 groups, only the LTD group had a statistically significant difference with not plasticity group (LTP group vs not plasticity group: $p=0.2$; LTD group vs not plasticity group: $p<0.05$; LTD group vs LTP group: $p=0.1333$) (Figure 5.A y 5.B)

When we compared the final PPR with baseline PPR, they did not present a statistically significance difference: in LTP experiments basal PPR was 0.6412 and final was 0.6117. ($p>0.99$); in case of LTD, basal PPR was 0.5774 and final was 0.5975 ($p=0.875$); and in not plasticity group, the basal PPR was 0.6883 and final was 0.7863 ($p= 0.6250$). (Figure 5.C, 5.D y 5.E). The number of experiments need to be increased but thus far, does not support a presynaptic mechanism of plasticity expression.

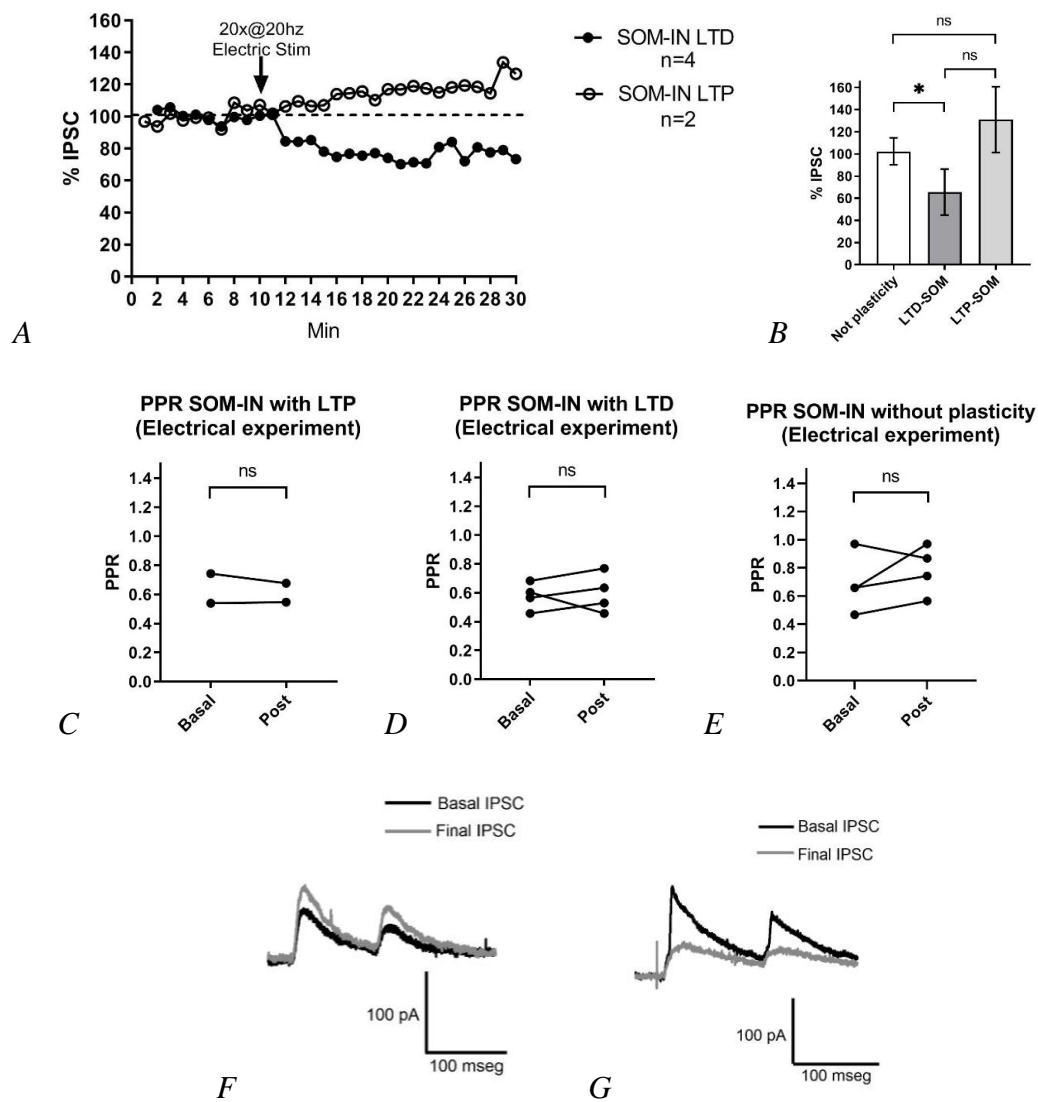


Figure 5. Effect of electrical stimulation at 20 Hz in IPSC mediated by SOM-IN. *A*: Time course of IPSCs mediated by SOM-INs: cases with LTP ($n=2$) in white circles, cases with LTD ($n=4$) in black circles. The black arrow shows when the protocol was applied. *B*: Comparison in percent of IPSC in LTP, LTD and not plasticity group. *C*: PPR change in each SOM-IN with LTP. *D*: PPR change in each SOM-IN with LTD. *E*: PPR change in each SOM-IN without plasticity. *F*: IPSC-LTP example in SOM-synapse. *G*: IPSC-LTD example in SOM-synapse ($n.s.$ = not significant, $* p = 0.0286$).

6.2.3 PV Experiments:

In case of PV experiments, the cells with inhibitory LTP (n=4) showed a mean increase of 111.5% in the amplitude of IPSC, with respect to the baseline; and the group with iLTD (n=6) had a mean decrease to 74.2% in the amplitude of IPSC. In the statistical difference, respect the baseline, only the group with iLTD had a significant difference ($p < 0.05$), but not the group with iLTP ($p = 0.125$). When we compared the group with iLTP and with iLTD, it existed as a statistically significant difference ($p < 0.05$) (Figure 6.A y 6.B).

In the PPR analyses, the mean baseline value of iLTP group was 0.769 while the final was 0.771, without statistically significant difference ($p = 0.625$). In contrast, in the iLTD group, on average the PPR showed a slight increase from 0.678 in baseline to 0.872 in final, with a statistically significant difference ($p < 0.05$) (Figure 6.D y 6.E). In the only case without plasticity also existed a little increase in PPR from 0.459 to 0.531, but we couldn't compare this value because only we obtained a unique case in this situation. Again, we cannot conclude without more experiments but thus far, our data suggest that LTD of PV-inhibition may be expressed presynaptically as a reduction in GABA release.

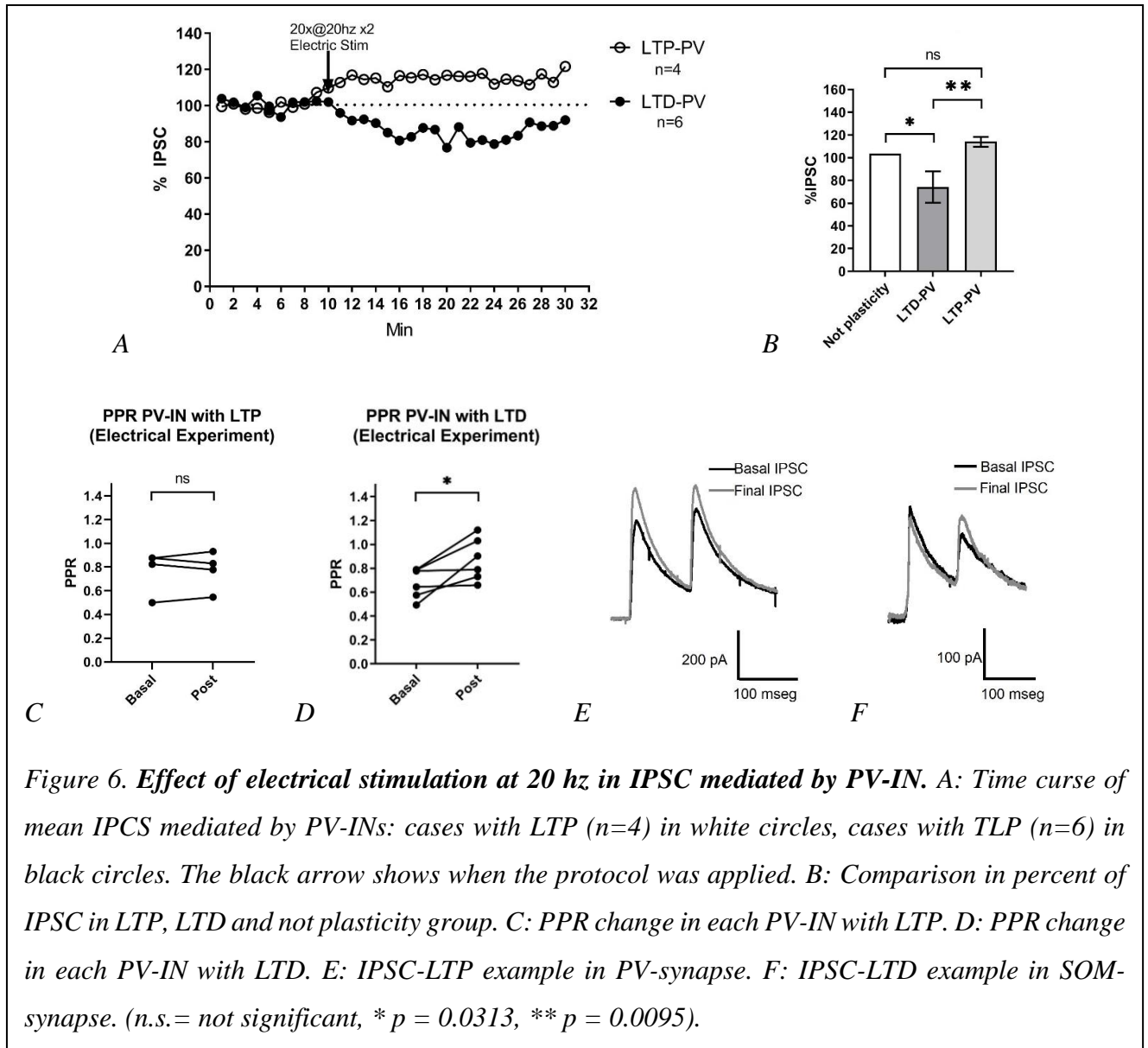


Figure 6. Effect of electrical stimulation at 20 Hz in IPSC mediated by PV-IN. **A:** Time course of mean IPSCs mediated by PV-INs: cases with LTP ($n=4$) in white circles, cases with LTD ($n=6$) in black circles. The black arrow shows when the protocol was applied. **B:** Comparison in percent of IPSC in LTP, LTD and not plasticity group. **C:** PPR change in each PV-IN with LTP. **D:** PPR change in each PV-IN with LTD. **E:** IPSC-LTP example in PV-synapse. **F:** IPSC-LTD example in SOM-synapse. (n.s. = not significant, * $p = 0.0313$, ** $p = 0.0095$).

6.3 Effect of optogenetic stimulation at 20 hz.

6.3.1 SOM Experiments

In the optogenetic experiments, we only studied SOM-cre mice injected previously with Chrimson conditional to cre virus in PFC, and ChR2 unconditional virus in MD thalamus. In the PV-cre animals injected by both viruses, the expression of non-conditional ChR2 was not enough for evoke a response from thalamus (in this case, we used those animals to electrical stimulation). 19 experiments were conducted evaluating the effect of optogenetic stimulation from thalamus at 20 hz in the IPSC from pyramidal cell in PFC. On average, after 20-Hz optogenetic stimulation, the amplitude of IPSCs was 92.5% respect his baseline, but without statistically significant difference ($p=0.1134$) (Figure 7.A y 7.C). The mean baseline PPR in those experiments was 0.72 and final 0.691 without statistically significant difference ($p=0.1134$) (Figure 7.D).

In all experiments evaluated, 10.5% (n=2) had iLTP, 42.1% (n=8) had iLTD, and 47.4% (n=9) did not present any change in the IPSC (Figure 9).

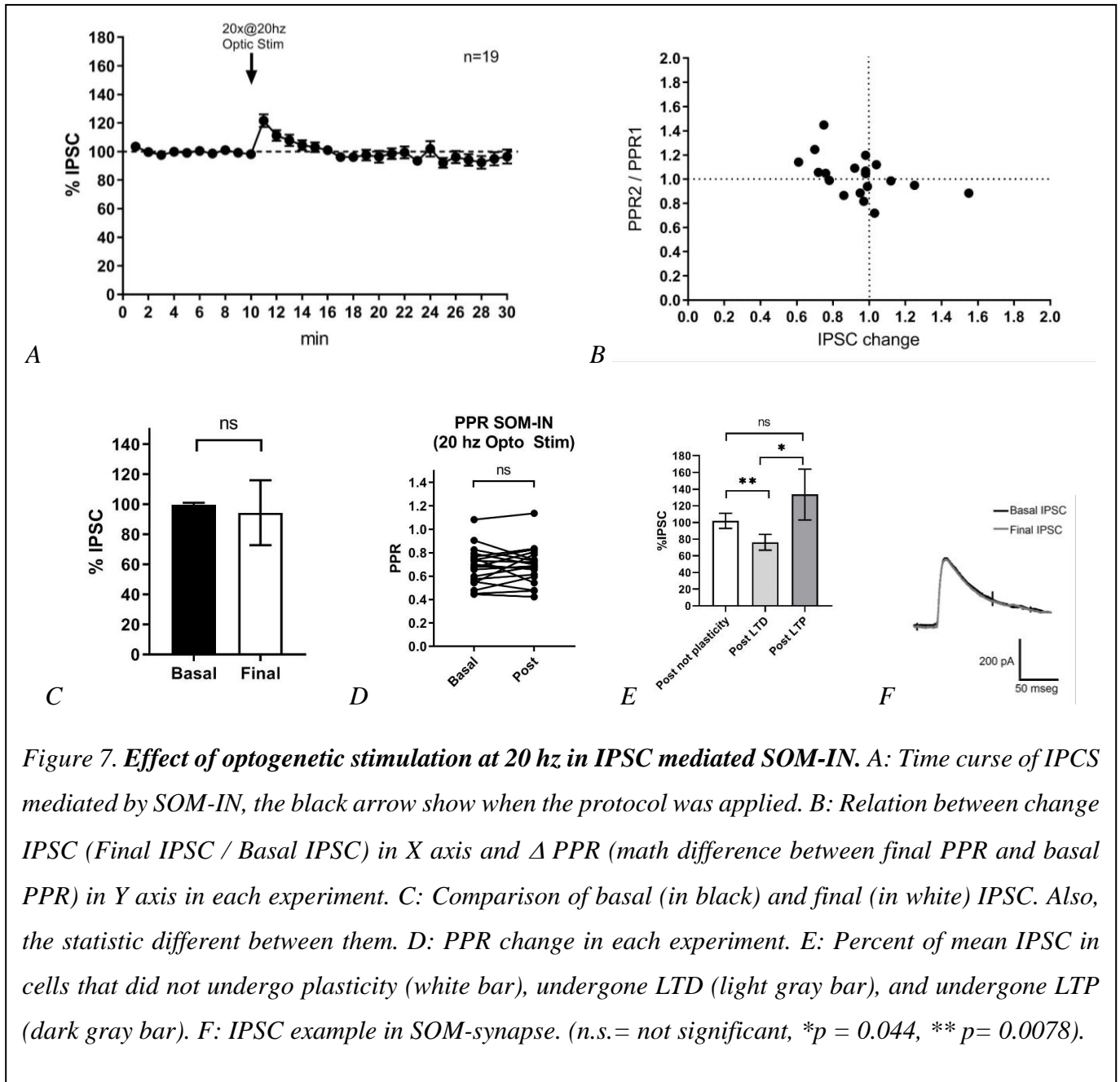


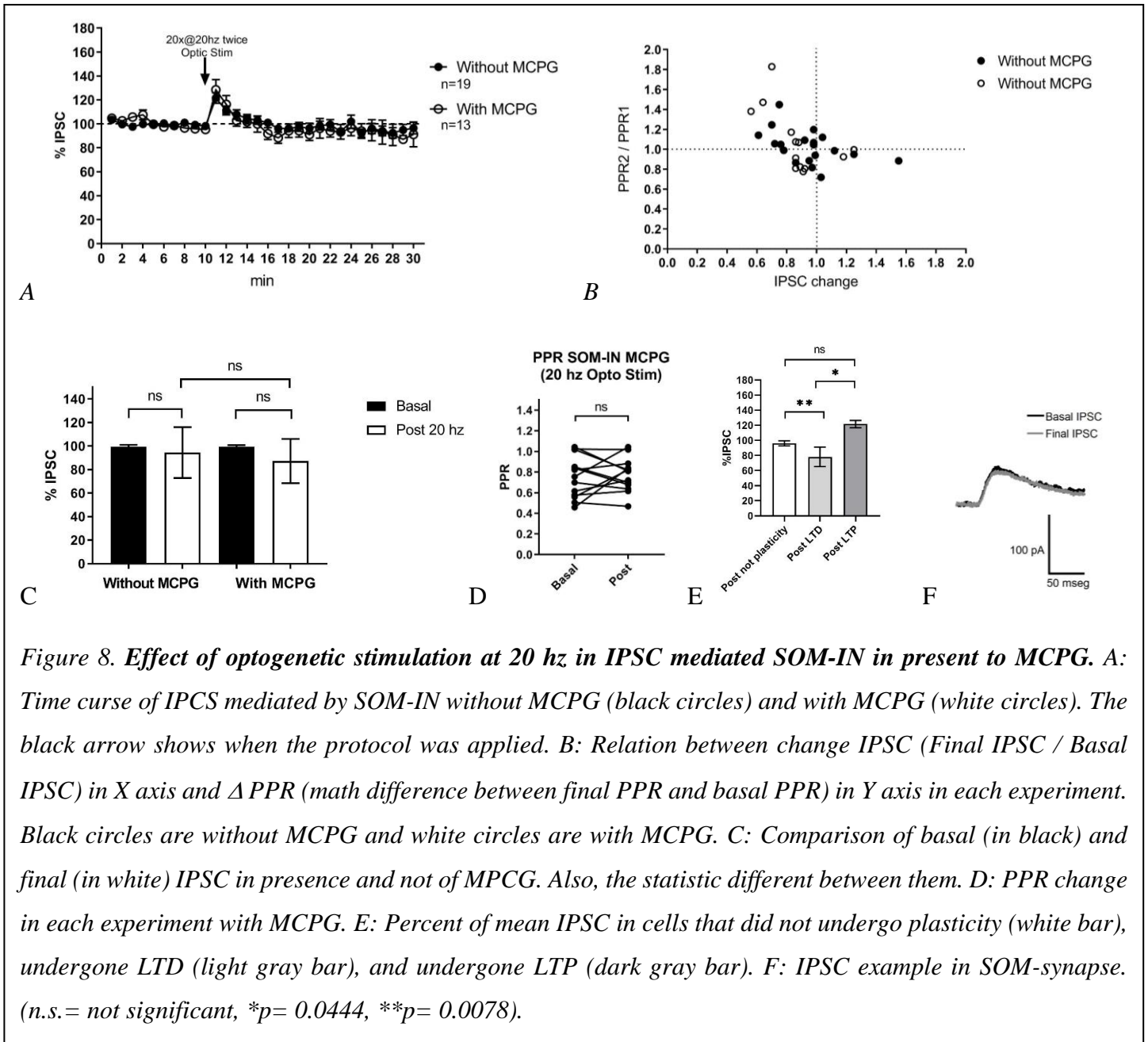
Figure 7. Effect of optogenetic stimulation at 20 Hz in IPSC mediated SOM-IN. *A: Time course of IPSCs mediated by SOM-IN, the black arrow shows when the protocol was applied. B: Relation between change in IPSC (Final IPSC / Basal IPSC) on the X-axis and Δ PPR (mathematical difference between final PPR and basal PPR) on the Y-axis in each experiment. C: Comparison of basal (in black) and final (in white) IPSC. Also, the statistical difference between them. D: PPR change in each experiment. E: Percent of mean IPSC in cells that did not undergo plasticity (white bar), underwent LTD (light gray bar), and underwent LTP (dark gray bar). F: IPSC example in SOM-synapse. (n.s. = not significant, * $p = 0.044$, ** $p = 0.0078$).*

6.3.2 SOM Experiments with MCPG in bath.

More than 50% of experiments showed a change in their amplitude after optogenetic 20 hz stimulation. For that reason, we wanted to know the influence of metabotropic glutamatergic receptors (mGluRs) in this process. We replicated the 20 hz optogenetic stimulation experiment with MCPG (antagonist of mGluR) in the bath. 13 experiments were done to evaluate this.

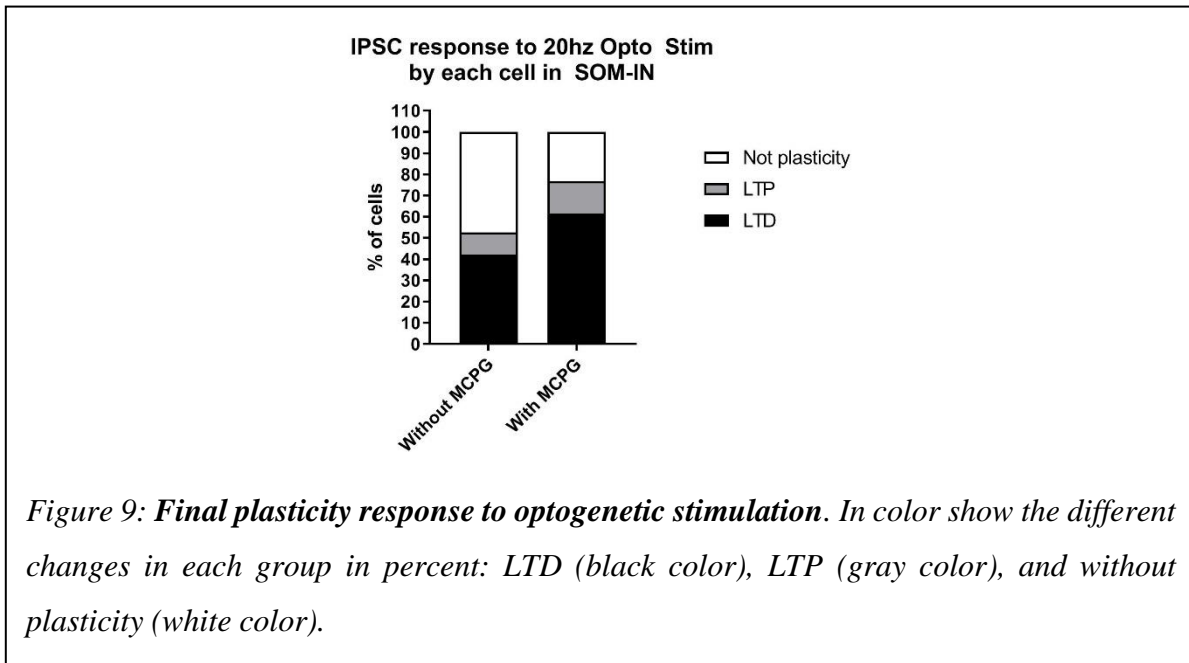
On average, the final amplitude of IPSC was 91.71%, with respect to the basal IPSC. No statistically significant difference was found between basal and final IPSC ($p=0.0681$) (Figure 8.A y 8.C). The PPR analyzes show a slight decrease of the value from 0.792 to 0.717 without a statistically significant difference ($p=0.839$) (Figure 8.D).

After this protocol, in all experiments, the 15.4% ($n=2$) had iLTP, 61.5% ($n=8$) showed iLTD and 23.1% ($n=3$) did not experiment any change in the amplitude (Figure 9). Thus preliminarily, mGluR blockade slightly increased the probability of inducing iLTD and probably does not contribute to iLTD as we postulated initially. We analyze the magnitude of plasticity and PPR in the following section.

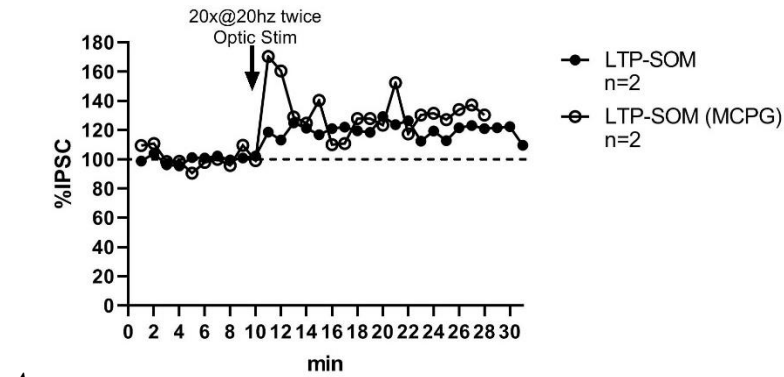


6.3.3 Analyze by group.

As we showed in the previous section, the 20hz optogenetic stimulation protocol did manage to induce plasticity in a percent of population inhibitory synapses in ACSF and when we added MCPG to ACSF (even better than without the drug) (Figure 9). For these reasons we will show the analyses of LTP and LTD groups.

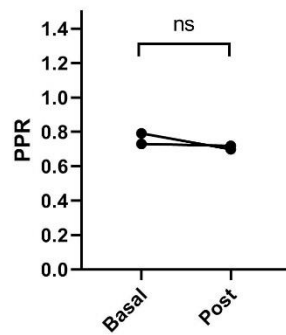


The synapses with LTP (n=2, in both cases) showed a mean increase to 133.5% in absence of MCPG, and 121.5% in group with MCPG (Figure 10.A). A statistically non-significant difference was found between them ($p > 0.999$) (Figure 12). On average, in the group without MCPG, the basal PPR was 0.761 and final was 0.71. When MCPG was added, similar values of PPR were obtained with a baseline of 0.765 and final of 0.743. In both cases, a non-statistically significant difference was found with respect to their basal ($p = 0.333$ and $p = 0.667$, respectively) (Figure 10.B and y 10.C).



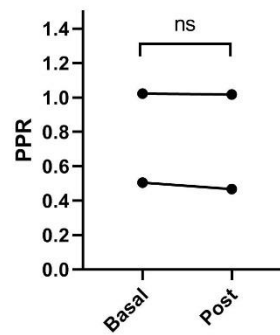
A

PPR SOM-IN with LTP
(optogenetic experiment)



B

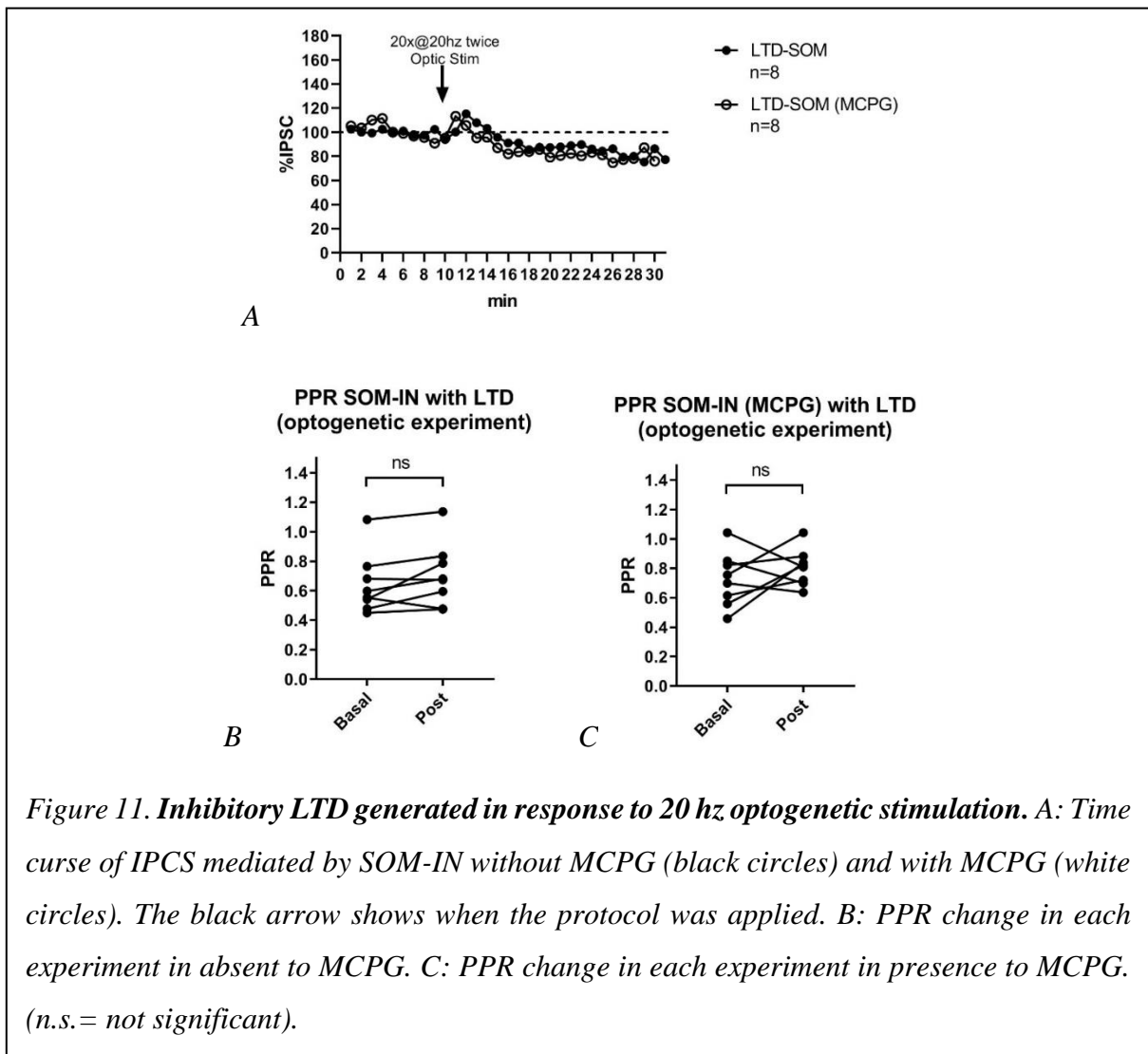
PPR SOM-IN (MCPG) with LTP
(optogenetic experiment)



C

Figure 10. Inhibitory LTP generated in response to 20 hz optogenetic stimulation. A: Time course of IPSCs mediated by SOM-IN without MCPG (black circles) and with MCPG (white circles). The black arrow shows when the protocol was applied. B: PPR change in each experiment in absence of MCPG. C: PPR change in each experiment in presence of MCPG. (n.s.= not significant).

In case of inhibitory LTD (n=8, in both cases), the final average amplitude in the group without MCPG was 76.25%, and in the group with MCPG was 78.13% (Figure 11.A). There was no statistically difference between them (p=0.7413) (Figure 12). Respect the PPR, the mean basal in the first group was 0.644 and the mean final was 0.71. In the group in presence to MCPG, the basal PPR was 0.723 and final was 0.81. Despite the slight difference between the basal and final PPR in both groups, a non-statistically significant difference was found between them (p=0.109 and p=0.3828, respectively) (Figure 11.B y 11.C).



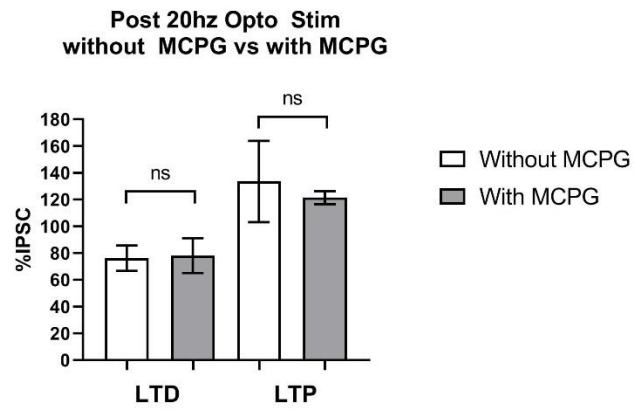


Figure 12: Comparison inhibition LTD and LTP in absent and presence of MCPG. White bars correspond to plasticity in absent MCPG, gray bars correspond to plasticity in presence MCPG. (n.s. = not significant).

7.- DISCUSSION:

A purpose of this work was to show differences in the plasticity changes of inhibitory synapse mediated by SOM-IN and PV-IN. First the idea was corroborating the inhibitory plasticity when NMDARs are activated, and then the effect of endogenous glutamate in the synapse through fibers of layer 4 stimulation by electrical protocol and fibers from MD thalamus by optogenetic stimulation.

In this way, we found NMDA induces a change in inhibitory synapse from SOM-IN, but not from PV-IN: IPSC mediated by SOM-IN increased the amplitude to 138.48% after exogenous NMDA application. NMDAR is an ionotropic glutamatergic receptor present in excitatory and inhibitory synapse, and it has been reported its participation in plasticity phenomena of both circuits (Moreau and Kullmann, 2013). Inhibitory plasticity by NMDAR involves an interaction between excitation and inhibition synapses: excitatory synapse must occur to induce heterosynaptic plasticity in GABAergic synapse via CaMKII signaling. CaMKII, on the other hand, is selectively translocated in dendrites of neurons (Chiu et al., 2019). In inhibitory synapse, the contribution of NMDAR has been reported in different studies: increase of GABA release by activation presynaptic NMDA in cerebellar interneuron, multipolar interneuron and stellate interneurons; postsynaptic NMDAR-dependent iLTP/D in visual cortex, hippocampus, cerebellar nucleus, ventral tegmental area (dopaminergic neurons) and brainstem (Moreau and Kullmann, 2013). Chiu et al in 2018 explored the influence of NMDAR activation in inhibitory synapses of different GABAergic neurons of PFC. Only synapses from SOM-IN to pyramidal cells had iLTP with an increase of IPSC to $171 \pm 18\%$, but not from PV-IN and VIP-IN. The iLTP was blocked by MK801 (internal antagonist of NMDAR), BAPTA (internal Ca^{+2} quelator), and KN-62 and IAP (external and internal block of CaMKII α , respectively), concluding that iLTP is induced by postsynaptic NMDAR, needs internal Ca^{+2} rise, and requires CaMKII activation. The lack of plasticity in the PV-IN synapse could be because perisomatic synapse has a lower sensibility to CaMKII α than dendritic synapse, and it was verified by postsynaptic addition of CamKII, Ca^{+2} and calmodulin while evaluating perisomatic synapse. This investigation and us provide background to say that the constitution of synapse would differ in the dendritic tree and this iLTP could be explained by the proximity of glutamatergic inputs in distal dendrite (Chiu et

al., 2018). The NMDAR activation in cultured neuron of hippocampus increase GABA_AR in postsynaptic dendrites, and along with this, in the amplitude of miniature IPSC (mIPSC), through CaMKII α activation (Moreau and Kullmann, 2013, Bannai et al., 2009). This can be explained by an increase GABA_AR mobility and receptor cluster dispersal due to Ca⁺²/calcineurin-dependent dephosphorylation within the intracellular loop of GABA_AR γ 2 subunit (Moreau and Kullmann, 2013, Muir et al., 2010). Marsden et al. studied the effect of NMDA in GABA_AR expression of neuron of hippocampus measuring the fluorescent GABA α 2/3 subunit (previously adding antibody to this subunit) and comparing the change of this after NMDA. The surface fluorescence increased by $67.2 \pm 12.1\%$ after NMDA, indicating an increase in the number of surface GABA_ARs. This change in the GABA_AR expression required a Ca⁺² rise and CaMKII activation (blocked by BAPTA-AM and KN-93, respectively). Simultaneously, $35.15 \pm 4.72\%$ decrease was generated in the fluorescence of surface AMPAR GluR1 subunit (using double marking). When they explored mIPSC mediated by GABA_AR, after NMDA, the amplitude and frequency were increasing. This was blocked by botulinum toxin light chain type B (BoNT/B). Using BAPTA and CaMKII AIP together in recording pipette the change in mIPSC amplitude was blocked, but not the frequency. This corroborates that this process involves exocytosis, and that it requires Ca⁺² rise and CaMKII (Marsden et al., 2007). Like our results, the PPR of inhibitory synapse did not change after NMDA, indicating a postsynaptic process. The inhibitory presynaptic plasticity has been more characterized compared by inhibitory postsynaptic plasticity. Petrini et al investigated how the GABA_AR traffic is after iLTP induced by NMDA in the hippocampus. Like other studies mentioned before, NMDA generated an increase in amplitude of mIPSC, spontaneous IPSC (sIPSC) and evoked IPSC (eIPSC), which was prevented by KN-62 and BAPTA (internal solution), concluding a postsynaptic mechanism. In this iLTP, diffusion coefficient of GABA_AR was reduced, while lateral mobility from extrasynaptic space was not modified, suggesting that it was selective to synaptic receptors, which would be immobilized in the synapse by a CaMKII-dependent mechanism. They used mutant CaMKII-K42R, the effect of NMDA was blocked. Also, this process involves accumulation of gephyrin protein thanks GABA α 3^{S383} phosphorylation by CaMKII; the main effect of gephyrin in GABA_AR mobility is to keep trapping of the receptor in synapse without modifying its intrinsic properties (Petrini et al., 2014). Although it possibly was not our case,

NMDAR activation may also participate in presynaptic plasticity allowing retrograde nitric oxide (NO) release that increase GABA release from terminal (Xue et al., 2011), and in other hand, it can favor the retrograde endocannabinoid release to reduce GABA release from terminal (Szabadits et al., 2011).

In next experiments, we tried to replicate the effect previously induced by NMDAR activation, but this time using endogenous neurotransmitter for its activation and in more physiological context. Medial dorsal (MD) thalamus of mice makes strong synaptic connection with prefrontal cortex (PFC), having preference with medial PFC (mPFC), where the experiments was carried out. In mice exists reciprocal activity between mPFC and MD thalamus for spatial working memory: MD inputs to mPFC participate in working memory maintenance, and mPFC inputs to MD support recovery of memory for action execution of actions (Bolkan et al., 2017). Electrical stimulation electrode was put in layer 4 of mPFC because this is the principal entry site for thalamic input and the stimulation protocol was at 20 hz because is thalamic trigger in vivo (Steriade et al., 1991, Leresche et al., 2012).

After 20 hz electrical stimulation, in average, inhibitory synapse mediated by SOM-IN and PV-IN did not show change in amplitude. Interestingly, PPR of PV-synapse increased, which could be indicating presynaptic modifications. In SOM-synapse, this protocol induced long term plasticity in 60% of the cases evaluated (n=6), while in PV-synapse it was induced in 90.9% of the cases (n=10). Also, the PV-synapse with iLTD (n=6) showed an increase of PPR. This suggests the PV-synapse was more susceptible to electrical protocol than SOM-synapse. These results were not similar to those found with NMDA protocol. First, chemical protocol with NMDA could activate synaptic and extrasynaptic NMDAR, and LTP can be consequence of this situation. Maybe electrical protocol cannot induce enough glutamate to act on both receptors. Other important difference is that the stimulation electrode generates an electrical pulse that activates all neurons near to the electrode, which can activate glutamatergic terminal from MD or other areas, excitatory neurons and interneurons. For that reason, the results are unspecific. Plastic changes associated with modifications in the membrane potential via VGCC have been reported in perisomatic inhibition of pyramidal cell of layer 5: high frequency of AP induces iLTP by Ca^{+2} rise through type R VGCC, while its hyperpolarization induces iLTD by Ca^{+2} rise through type L VGCC. Both blocked by botulinum toxin,

suggesting a change in the number of postsynaptic GABA_AR (Kurotani et al., 2008, Chiu et al., 2019), which could be a possibility in our case because in the majority of plastic changes obtained PPR did not change. Both inhibitory synapses presented an important proportion of iLTD (40 % of SOM-synapse; 54.5% of PV-synapse). One possibility is via eCBs In hippocampus. In hippocampus, the mechanisms involve in endocannabinoids synthesis are in pyramidal cell dendrites (DGL α and mGluR5 more expressed here), but CB1R was similar expressed in perisomatic and dendritic GABAergic terminal. The effect of endocannabinoids was compared between dendritic synapse by CCK-positive Schaffer collateral-associated cells and perisomatic synapse by CCK-positive basket cells. eCB-mediated stronger somatic input than dendritic input, which was corroborated through chemical activation of mGluR1 (DHPG) and CB1R (WIN). Anyway, IPSC could be blocked when high concentration of DHPG or WIN was used, suggesting presynaptic functional differences downstream from CB1R in both GABAergic synapses (Lee et al., 2010). The activation of mGluR1 of CA1 pyramidal cells, driving retrograde endocannabinoids synthesis. Presynaptic CB1R by endocannabinoid reduces GABA release. This requires cAMP-PKA and calcineurin signals, and active zone protein RIM1 α (Chiu et al., 2019).

With optogenetic stimulation, we want to evoke a change by MD thalamus terminals to induce glutamatergic release in layer 4 of mPFC. In the SOM-pyramidal cell synapse, after 20 hz stimulation of glutamatergic inputs, 52.62% of cell evaluated (n= 10) presented long-term plasticity and mostly it was iLTD (n=8). Similar to electrical stimulation, the released glutamate can act on pyramidal cells directly (mGluR, NMDAR, AMPAR) or GABAergic neurons favoring GABA release thought disinaptic mechanisms. To explore if cases with iLTD could be thanks to endocannabinoid, we used MCPG in external solution to block mGluR. With this, 76.9% of cell evaluated experimented long-term plasticity (n=10), being mostly iLTD. This suggests little or no contribution of eCB signaling or if eCBs are involved, it does not require mGluRs. This needs to be resolved with CB1R antagonism. Another possibility is GABA_BR activation. GABA_BR is a G_{i/o} protein coupled receptor present in presynaptic and postsynaptic surfaces, generating slower and longer inhibition than GABA_AR. It is made up by GABA_B1 (ligand-binding domain) and GABA_B2 (coupled to G protein) subunits (Kantamneni et al., 2014). GABA can act on presynaptic or postsynaptic cells, and depending on whether it is about excitatory or inhibitory synapse, are the effects (Guetg et al.,

2009, Kantamneni et al., 2014). Presynaptic GABA_BR activation reduces neurotransmitter release through inhibition of VGCC, inducing inhibition of synapse (in glutamatergic terminal) or disinhibition (in GABAergic terminal). In thalamus and hippocampus, the activation of presynaptic GABA_BR in inhibitory synapse requires a strong stimulation, through a synchronous activity (Sanchez-Vives et al., 2021) and we suspect this mechanism could be involved in the iLTD-PV, because the electrical stimulation generates a big activation of all cellular groups near to electrode. To this is added, the change in PPR is representative of a presynaptic process.

Postsynaptic GABA_BRs are widely localized in spines and dendrites. Here, they active inwardly rectifying K⁺ channels and reduce Ca⁺² signals in dendrite and dendritic arbor. This direct action on the spines allows them modulate LTP (Chalifoux and Carter, 2011, Sanchez-Vives et al., 2021). The opening of K⁺ channels increase membrane conductance and produces slow IPSP, which can suppress postsynaptic response and AP backpropagated. Using two-photon microscopy, Chalifoux et al. measured Ca⁺² signals triggered by somatic AP backpropagated in dendritic arbor in layer 2/3 pyramidal cell of PFC, in different context that involves GABA_BR activation. Using BACLOFEN (GABA_BR agonist), they confirmed a decreasing of the Ca⁺² signals in over dendritic arbor when GABA_BRs are activated. After that, they used puffing BACLOFEN, and their results suggested apical dendrites could have GABA_BR and K⁺ channels. Using internal Cs and TTX, the reduction in Ca⁺² signals was maintained suggesting the participation of VGCCs. T-type, L-type, R-type and P/Q/BNtype VGCCs was blocked by drugs, confirming their participation in the decrease of Ca⁺² signals after GABA_BR activation (Chalifoux and Carter, 2011). More evidence has been found about this. In apical tuft dendrites, GABA_BR inhibit postsynaptic VGCC Ca⁺² signals (Chalifoux and Carter, 2011, Perez-Garci et al., 2006), while in basal spines GABA_BR inhibit postsynaptic NMDAR Ca⁺² signals (Chalifoux and Carter, 2010). In our investigation, the inhibition of VGCC by GABA_BR activation is a possibility of iLTD-SOM found. In other aspect, this receptor is abundant at excitatory synapses, where it interacts reciprocally with NMDAR: Selective activation of synaptic NMDAR improves GABA_{B1} and GABA_{B2} surface expression (increased by 75.4 ± 17.2.% and 55.7 ± 4.3%, respectively compared with basal), but when extrasynaptic NMDARs are activated, both subunits decrease their expression (decrease by 79.3 ± 4% and 86.8 ± 3.3%, respectively compared with basal). This

was proved in cultured hippocampal neurons. In this study, it is mentioned that NMDAR activation control surface expression of GABA_BR via regulation of the cycling endosome (Kantamneni et al., 2014).

Interestingly, the optogenetic protocol induced short-term potentiation that could not be induced by electrical protocol. Short-term potentiation in this case corresponds to the post-tetanic potentiation (PTP). PTP is a short-term plasticity observed following high-frequency synaptic activation that activates VGCC in terminal with presynaptic Ca⁺² rise and involves an increase in the probability of release (Fioravante and Regehr, 2011). This has been reported in cultured rat hippocampal neurons that showed PTP in IPSC after 20 hz and 80 hz presynaptic stimulation (Jensen et al., 1999). Like us, optogenetic stimulation at 20 hz evoked PTP in IPSC, so we can say that thalamic input stimulation generated stronger disynaptic process. In contrast, is possible that electrical stimulation could not induce enough Ca⁺² rise in presynaptic inhibitory terminal to generated PTP.

Other differences between SOM-synapse and PV-synapse have been reported. In mPFC the expression of β 2/3 GABA_AR is functionally enriched in SOM-synapse, respect PV-synapse, because with β 2/3-selective positive allosteric modulator (ETOMIDATE) only decay of IPSC mediated by SOM was slowed, but not IPSC mediated by PV-IN (Chiu et al., 2018). Structurally, deletion of Neuroglin 2 molecule (protein involves in maintaining structure and functionality of inhibitory synapse) worsens PV-synapse, but not in SOM-synapse, suggesting that Neuroglin 2 may play different role in both interneuron types (Gibson et al., 2009). Functionally, the activity of postsynaptic pyramidal cells preferably modifies the perisomatic inhibition, respect to dendritic. In layer 5 of somatosensory cortex of mice, using AP stimulation (current clamp mode) in pyramidal cell (5 AP at 100 hz), perisomatic IPSP had an iLTP (basal: 1.42 ± 0.23 mV; after AP protocol: 2.9 ± 0.57 mV), but distal IPSP was not modified. The same occurs with depolarization protocol (ten 5-s long steps to 0 mV, repeated every 30) that induced an increase at $59.18 \pm 24.63\%$ in IPSC mediated by PV-IN. This process would be presynaptic, involves postsynaptic Ca⁺² rise (was blocked by BAPTA) depending on type L VGCC (blocked by NIFEDEPINE) and retrograde release of NO (blocked by L-NAME or ODQ). NO also can spread to near synapses and induces subtle iLTP in the neighbors. Using SNAP, they showed that dendritic inhibitory synapse was insensitive

to NO possibly because NO receptor guanylylcyclase (GC) is preferably expressed in perisomatic terminal of PV-IN (Lourenco et al., 2014). Other experiments in the same cortex, but in neurons of layer 2/3 using TTX to block chronic activity, was performed to see the effect of this condition in synapses (including SOM and PV-synapse). AP train (8 AP at 20 Hz) of interneurons did not generate change in amplitude of IPSC mediated by SOM or PV, but it did induce decrease in percent connected of all cell pairs tested for IPSC-PV, along with this, a 30% decrease in local inhibitory drive provided by PV-IN. SOM-synapse did not have any of the above modifications. Short stimulation trains were used to value short-term plasticity. PV-IN circuit did not change, but SOM-IN circuit did: IPSC-SOM became less depressing (or more facilitator). The conclusion in this case is when activity of circuit is blocked, there is change in contribution of inhibition mediated by both interneurons types: SOM-IN contributes more with a low stimulation frequency, and PV-IN with a quick stimulation frequency (Bartley et al., 2008). In layer 2/3 of the visual cortex of mice, upregulation or down regulation of activity led to increasing or decreasing of perisomatic inhibition by PV-IN, but without change in dendritic inhibition by SOM-IN. This could be explained by retrograde release of NO from postsynaptic cells (Xue et al., 2014, Chiu et al., 2019). Retrograde BDNF could induce iLTP mainly in PV-synapse, because its receptor (TRKB) is preferably expressed in axonal terminal of PV-IN to modulate Ca^{+2} dependent GABA release (Sun et al., 2014, Chiu et al., 2019).

To try to elucidate the effects of glutamatergic inputs from MD thalamus, we propose to carry out the experiment with NMDAR, GABA_BR and VGCC antagonists. Depending on these results, consider using Ca^{+2} a chelator to prevent Ca^{+2} rise; or TTX to prevent AP. It is recommended to use the double optogenetic focus for more information regarding physiological neurological activity. We believe it is important to mention that the use of double optogenetic technique was hard, because we needed the virus expression in two zones: mPFC and terminals from MD thalamus. The optogenetic channel did not express in all terminals from MD thalamus that we injected. Because of that, we could not perform this experiment in PV-cre mice. In some cases, we obtained expression to the channels, but it was not enough to induce response with light stimulation.

8.- CONCLUSION

NMDAR activation modifies preferably the synapse mediated by SOM-IN (dendritic), inducing inhibitory long-term potentiation. This would involve postsynaptic mechanisms because PPR was not changed. In contrast, NMDA did not affect synapse mediated by PV-IN. The described process by which NMDAR induces postsynaptic iLTP requires Ca^{+2} rise and CaMKII activation.

For other hand, 20 hz stimulation in layer 4 of mPFC by electrical electrode or glutamatergic input from MD thalamus (optogenetic protocol) induces varied plastic changes at inhibitory synapse. Both electrical and optogenetic stimulation can activate different neuronal groups and along with this, induce changes by different signaling cascades, including disynaptic processes, which could explain our results. This would have greater impact in synapse mediated by PV-IN. Inhibitory long-term depression was the plastic change more frequently after 20 hz protocol. About this, we suspect GABA_BR activation would be involved in the mechanism. In case of synapse by SOM-IN, this did not include PPR change, compared to synapse by PV-IN, which did have PPR change, suggesting presynaptic change.

9. BIBLIOGRAPHY

- ADESNIK, H., BRUNS, W., TANIGUCHI, H., HUANG, Z. J. & SCANZIANI, M. 2012. A neural circuit for spatial summation in visual cortex. *Nature*, 490, 226-31.
- ALONSO, J. M. & SWADLOW, H. A. 2005. Thalamocortical specificity and the synthesis of sensory cortical receptive fields. *J Neurophysiol*, 94, 26-32.
- BANNAI, H., LEVI, S., SCHWEIZER, C., INOUE, T., LAUNEY, T., RACINE, V., SIBARITA, J. B., MIKOSHIBA, K. & TRILLER, A. 2009. Activity-dependent tuning of inhibitory neurotransmission based on GABAAR diffusion dynamics. *Neuron*, 62, 670-82.
- BANNON, N. M., CHISTIANKOVA, M. & VOLGUSHEV, M. 2020. Synaptic Plasticity in Cortical Inhibitory Neurons: What Mechanisms May Help to Balance Synaptic Weight Changes? *Front Cell Neurosci*, 14, 204.
- BARTLEY, A. F., HUANG, Z. J., HUBER, K. M. & GIBSON, J. R. 2008. Differential activity-dependent, homeostatic plasticity of two neocortical inhibitory circuits. *J Neurophysiol*, 100, 1983-94.
- BEIERLEIN, M., GIBSON, J. R. & CONNORS, B. W. 2003. Two dynamically distinct inhibitory networks in layer 4 of the neocortex. *J Neurophysiol*, 90, 2987-3000.
- BIENENSTOCK, E. L., COOPER, L. N. & MUNRO, P. W. 1982. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J. Neurosci*, 2, 32-48.
- BITTNER, S. R., WILLIAMSON, R. C., SNYDER, A. C., LITWIN-KUMAR, A., DOIRON, B., CHASE, S. M., SMITH, M. A. & YU, B. M. 2017. Population activity structure of excitatory and inhibitory neurons. *PLOS ONE*, 12.
- BOLKAN, S. S., STUJENSKE, J. M., PARNAUDEAU, S., SPELLMAN, T. J., RAUFFENBART, C., ABBAS, A. I., HARRIS, A. Z., GORDON, J. A. & KELLENDONK, C. 2017. Thalamic projections sustain prefrontal activity during working memory maintenance. *Nat Neurosci*, 20, 987-996.
- CAPUTI, A., ROZOV, A., BLATOW, M. & MONYER, H. 2009. Two calretinin-positive GABAergic cell types in layer 2/3 of the mouse neocortex provide different forms of inhibition. *Cereb Cortex*, 19, 1345-59.
- CASTILLO, P. E., CHIU, C. Q. & CARROLL, R. C. 2011. Long-term plasticity at inhibitory synapses. *Curr Opin Neurobiol*, 21, 328-38.
- CRUIKSHANK, S. J., LEWIS, T. J. & CONNORS, B. W. 2007. Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. *Nat Neurosci*, 10, 462-8.

- CHALIFOUX, J. R. & CARTER, A. G. 2010. GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. *Neuron*, 66, 101-113.
- CHALIFOUX, J. R. & CARTER, A. G. 2011. GABAB receptor modulation of voltage-sensitive calcium channels in spines and dendrites. *J Neurosci*, 31, 4221-32.
- CHEVALEYRE, V., TAKAHASHI, K. A. & CASTILLO, P. E. 2006. Endocannabinoid-Mediated Synaptic Plasticity in the CNS. *The Annual Review of Neuroscience*, 29, 37-76.
- CHISTIAKOVA, M., ILIN, V., ROSHCHIN, M., BANNON, N., MALYSHEV, A., KISVARDAY, Z. & VOLGUSHEV, M. 2019. Distinct Heterosynaptic Plasticity in Fast Spiking and Non-Fast-Spiking Inhibitory Neurons in Rat Visual Cortex. *J Neurosci*, 39, 6865-6878.
- CHIU, C. Q., BARBERIS, A. & HIGLEY, M. J. 2019. Preserving the balance: diverse forms of long-term GABAergic synaptic plasticity. *Nature Reviews Neuroscience*, 20, 272-281.
- CHIU, C. Q., MARTENSON, J. S., YAMAZAKI, M., NATSUME, R., SAKIMURA, K., TOMITA, S., TAVALLIN, S. J. & HIGLEY, M. J. 2018. Input-Specific NMDAR-Dependent Potentiation of Dendritic GABAergic Inhibition. *Neuron*, 97, 368-377 e3.
- CHIU, C. Q., PUENTE, N., GRANDES, P. & CASTILLO, P. E. 2010. Dopaminergic modulation of endocannabinoid-mediated plasticity at GABAergic synapses in the prefrontal cortex. *J Neurosci*, 30, 7236-48.
- DIANA, M. A. & MARTY, A. 2004. Endocannabinoid-mediated short-term synaptic plasticity: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). *Br J Pharmacol*, 142, 9-19.
- FELDMEYER, D., QI, G., EMMENEGGER, V. & STAIGER, J. F. 2018. Inhibitory interneurons and their circuit motifs in the many layers of the barrel cortex. *Neuroscience*, 368, 132-151.
- FERNANDEZ-ALACID, L., AGUADO, C., CIRUELA, F., MARTIN, R., COLON, J., CABANERO, M. J., GASSMANN, M., WATANABE, M., SHIGEMOTO, R., WICKMAN, K., BETTLER, B., SANCHEZ-PRIETO, J. & LUJAN, R. 2009. Subcellular compartment-specific molecular diversity of pre- and post-synaptic GABA-activated GIRK channels in Purkinje cells. *J Neurochem*, 110, 1363-76.
- FIORAVANTE, D. & REGEHR, W. G. 2011. Short-term forms of presynaptic plasticity. *Curr Opin Neurobiol*, 21, 269-74.
- FISHELL, G. & RUDY, B. 2011. Mechanisms of inhibition within the telencephalon: "where the wild things are". *Annu Rev Neurosci*, 34, 535-67.

- FU, Y., TUCCIARONE, J. M., ESPINOSA, J. S., SHENG, N., DARCY, D. P., NICOLL, R. A., HUANG, Z. J. & STRYKER, M. P. 2014. A cortical circuit for gain control by behavioral state. *Cell*, 156, 1139-1152.
- GANDAL, M. J., SISTI, J., KLOOK, K., ORTINSKI, P. I., LEITMAN, V., LIANG, Y., THIEU, T., ANDERSON, R., PIERCE, R. C., JONAK, G., GUR, R. E., CARLSON, G. & SIEGEL, S. J. 2012. GABAB-mediated rescue of altered excitatory-inhibitory balance, gamma synchrony and behavioral deficits following constitutive NMDAR-hypofunction. *Transl Psychiatry*, 2, e142.
- GARKUN, Y. & MAFFEI, A. 2014. Cannabinoid-dependent potentiation of inhibition at eye opening in mouse V1. *Front Cell Neurosci*, 8, 46.
- GIBSON, J. R., HUBER, K. M. & SUDHOF, T. C. 2009. Neuroligin-2 Deletion Selectively Decreases Inhibitory Synaptic Transmission Originating from Fast-Spiking but Not from Somatostatin-Positive Interneurons. *Journal of Neuroscience*, 29, 13883-13897.
- GONCHAR, Y. & BURKHALTER, A. 2003. Distinct GABAergic targets of feedforward and feedback connections between lower and higher areas of rat visual cortex. *J. Neurosci*, 23, 10904-10912.
- GRIGUOLI, M., CELLOT, G. & CHERUBINI, E. 2013. In hippocampal oriens interneurons anti-Hebbian long-term potentiation requires cholinergic signaling via alpha7 nicotinic acetylcholine receptors. *J Neurosci*, 33, 1044-9.
- GUETG, N., SEDDIK, R., VIGOT, R., TURECEK, R., GASSMANN, M., VOGT, K. E., BRAUNER-OSBORNE, H., SHIGEMOTO, R., KRETZ, O., FROTSCHER, M., KULIK, A. & BETTLER, B. 2009. The GABAB1a isoform mediates heterosynaptic depression at hippocampal mossy fiber synapses. *J Neurosci*, 29, 1414-23.
- HAINMUELLER, T., KRIEGLSTEIN, K., KULIK, A. & BARTOS, M. 2014. Joint CP-AMPA and group I mGlu receptor activation is required for synaptic plasticity in dentate gyrus fast-spiking interneurons. *Proc Natl Acad Sci U S A*, 111, 13211-6.
- HORN, M. E. & NICOLL, R. A. 2018. Somatostatin and parvalbumin inhibitory synapses onto hippocampal pyramidal neurons are regulated by distinct mechanisms. *Proc Natl Acad Sci U S A*, 115, 589-594.
- HUANG, S., HUGANIR, R. L. & KIRKWOOD, A. 2013. Adrenergic gating of Hebbian spike-timing-dependent plasticity in cortical interneurons. *J Neurosci*, 33, 13171-8.
- JENSEN, K., SKOVGAARD JENSEN, M. & LAMBERT, J. D. C. 1999. Post-tetanic potentiation of GABAergic IPSCs in cultured rat hippocampal neurones. *Journal of Physiology*, 519, 71-84.

- JIA, Y., YAMAZAKI, Y., NAKAUCHI, S., ITO, K. & SUMIKAWA, K. 2010. Nicotine facilitates long-term potentiation induction in oriens-lacunosum moleculare cells via Ca²⁺ entry through non-alpha7 nicotinic acetylcholine receptors. *Eur J Neurosci*, 31, 463-76.
- JONAS, P. & BURNASHEVT, N. 1995. Molecular Mechanisms Controlling Calcium Entry through AMPA-Type Glutamate Receptor Channels. *Neuron*, 15, 987-990.
- KAESER, P. S., DENG, L., CHAVEZ, A. E., LIU, X., CASTILLO, P. E. & SUDHOF, T. C. 2009. ELKS2alpha/CAST deletion selectively increases neurotransmitter release at inhibitory synapses. *Neuron*, 64, 227-39.
- KANTAMNENI, S. 2015. Cross-talk and regulation between glutamate and GABAB receptors. *Front Cell Neurosci*, 9, 135.
- KANTAMNENI, S., GONZALEZ-GONZALEZ, I. M., LUO, J., CIMAROSTI, H., JACOBS, S. C., JAAFARI, N. & HENLEY, J. M. 2014. Differential regulation of GABAB receptor trafficking by different modes of N-methyl-D-aspartate (NMDA) receptor signaling. *J Biol Chem*, 289, 6681-6694.
- KARNANI, M. M., JACKSON, J., AYZENSHTAT, I., TUCCIARONE, J., MANOOCHERI, K., SNIDER, W. G. & YUSTE, R. 2016. Cooperative Subnetworks of Molecularly Similar Interneurons in Mouse Neocortex. *Neuron*, 90, 86-100.
- KIM, D., JEONG, H., LEE, J., GHIM, J. W., HER, E. S., LEE, S. H. & JUNG, M. W. 2016. Distinct Roles of Parvalbumin- and Somatostatin-Expressing Interneurons in Working Memory. *Neuron*, 92, 902-915.
- KISVARDAY, Z. F., BEAULIEU, C. & EYSEL, U. T. 1993. Network of GABAergic large basket cells in cat visual cortex (area 18): implication for lateral disinhibition. *J. Comp. Neurol*, 327, 398-415.
- KOCH, C., POGGIO, T. & TORRE, V. 1983. Nonlinear interactions in a dendritic tree Localization, timing, and role in information processing. *Proc. Natl. Acad. Sci. USA*, 80, 2799-2802.
- KULLMANN, D. M., MOREAU, A. W., BAKIRI, Y. & NICHOLSON, E. 2012. Plasticity of inhibition. *Neuron*, 75, 951-62.
- KUROTANI, T., YAMADA, K., YOSHIMURA, Y., CRAIR, M. C. & KOMATSU, Y. 2008. State-dependent bidirectional modification of somatic inhibition in neocortical pyramidal cells. *Neuron*, 57, 905-16.
- LACHAMP, P. M., LIU, Y. & LIU, S. J. 2009. Glutamatergic modulation of cerebellar interneuron activity is mediated by an enhancement of GABA release and requires protein kinase A/RIM1alpha signaling. *J Neurosci*, 29, 381-92.

- LEE, S., KRUGLIKOV, I., HUANG, Z. J., FISHELL, G. & RUDY, B. 2013. A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nat Neurosci*, 16, 1662-70.
- LEE, S. H., FOLDY, C. & SOLTESZ, I. 2010. Distinct endocannabinoid control of GABA release at perisomatic and dendritic synapses in the hippocampus. *J Neurosci*, 30, 7993-8000.
- LEI, S. & MCBAIN, C. J. 2002. Distinct NMDA Receptors Provide Differential Modes of Transmission at Mossy Fiber-Interneuron Synapses. *Neuron*, 33, 921-933.
- LERESCHE, N., LAMBERT, R. C., ERRINGTON, A. C. & CRUNELLI, V. 2012. From sleep spindles of natural sleep to spike and wave discharges of typical absence seizures: is the hypothesis still valid? *Pflugers Arch*, 463, 201-12.
- LIEN, C. C., MU, Y., VARGAS-CABALLERO, M. & POO, M. M. 2006. Visual stimuli-induced LTD of GABAergic synapses mediated by presynaptic NMDA receptors. *Nat Neurosci*, 9, 372-80.
- LISMAN, J. 1989. A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proc. Natl. Acad. Sci. USA* 86, 9574-9578.
- LISMAN, J. E. 2001. Three Ca²⁺ plasticity differently: the LTP zone, the LTD zone and no man's land levels affect. *J. Physiol*, 532, 285.
- LIU, G. 2004. Local structural balance and functional interaction of excitatory and inhibitory synapses in hippocampal dendrites. *Nature Neuroscience*, 7, 373-379.
- LIU, S. J. & LACHAMP, P. 2006. The activation of excitatory glutamate receptors evokes a long-lasting increase in the release of GABA from cerebellar stellate cells. *J Neurosci*, 26, 9332-9.
- LIU, Y., ZHANG, L. I. & TAO, H. W. 2007. Heterosynaptic scaling of developing GABAergic synapses: dependence on glutamatergic input and developmental stage. *J Neurosci*, 27, 5301-12.
- LOURENCO, J., PACIONI, S., REBOLA, N., VAN WOERDEN, G. M., MARINELLI, S., DIGREGORIO, D. & BACCI, A. 2014. Non-associative potentiation of perisomatic inhibition alters the temporal coding of neocortical layer 5 pyramidal neurons. *PLoS Biol*, 12, e1001903.
- LU, J. T., LI, C. Y., ZHAO, J. P., POO, M. M. & ZHANG, X. H. 2007. Spike-timing-dependent plasticity of neocortical excitatory synapses on inhibitory interneurons depends on target cell type. *J Neurosci*, 27, 9711-20.
- MALENKA, R. C. & BEAR, M. F. 2004. LTP and LTD. *Neuron*, 44, 5-21.
- MARSDEN, K. C., BEATTIE, J. B., FRIEDENTHAL, J. & CARROLL, R. C. 2007. NMDA receptor activation potentiates inhibitory transmission through GABA receptor-associated protein-dependent exocytosis of GABA(A) receptors. *J Neurosci*, 27, 14326-37.

- MARTINA, M., VIDA, I. & JONAS, P. 2000. Distal Initiation and Active Propagation of Action Potentials in Interneuron Dendrites. *Science*, 287, 295–300.
- MOREAU, A. W. & KULLMANN, D. M. 2013. NMDA receptor-dependent function and plasticity in inhibitory circuits. *Neuropharmacology*, 74, 23-31.
- MUIR, J., ARANCIBIA-CARCAMO, I. L., MACASKILL, A. F., SMITH, K. R., GRIFFIN, L. D. & KITTLER, J. T. 2010. NMDA receptors regulate GABAA receptor lateral mobility and clustering at inhibitory synapses through serine 327 on the gamma2 subunit. *Proc Natl Acad Sci U S A*, 107, 16679-84.
- NESKE, G. T. & CONNORS, B. W. 2016. Distinct Roles of SOM and VIP Interneurons during Cortical Up States. *Front Neural Circuits*, 10, 52.
- PAN, B., HILLARD, C. J. & LIU, Q. S. 2008. D2 dopamine receptor activation facilitates endocannabinoid-mediated long-term synaptic depression of GABAergic synaptic transmission in midbrain dopamine neurons via cAMP-protein kinase A signaling. *J Neurosci*, 28, 14018-30.
- PANZANELLI, P., GUNN, B. G., SCHLATTER, M. C., BENKE, D., TYAGARAJAN, S. K., SCHEIFFELE, P., BELELLI, D., LAMBERT, J. J., RUDOLPH, U. & FRITSCHY, J. M. 2011. Distinct mechanisms regulate GABAA receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. *J Physiol*, 589, 4959-80.
- PEREZ-GARCI, E., GASSMANN, M., BETTLER, B. & LARKUM, M. E. 2006. The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca²⁺ spikes in layer 5 somatosensory pyramidal neurons. *Neuron*, 50, 603-16.
- PETRINI, E. M., RAVASENGA, T., HAUSRAT, T. J., IURILLI, G., OLCESE, U., RACINE, V., SIBARITA, J. B., JACOB, T. C., MOSS, S. J., BENFENATI, F., MEDINI, P., KNEUSSEL, M. & BARBERIS, A. 2014. Synaptic recruitment of gephyrin regulates surface GABAA receptor dynamics for the expression of inhibitory LTP. *Nat Commun*, 5, 3921.
- ROZOV, A. & BURNASHEV, N. 1999. Polyamine-dependent facilitation of postsynaptic AMPA receptors counteracts paired-pulse depression. *Nature*, 401, 594-598.
- RUDY, B., FISHELL, G., LEE, S. & HJERLING-LEFFLE, J. 2011. Three Groups of Interneurons Account for Nearly 100% of Neocortical GABAergic Neurons. *Developmental Neurobiology*, 71, 45-61.
- SAMBANDAN, S., SAUER, J. F., VIDA, I. & BARTOS, M. 2010. Associative plasticity at excitatory synapses facilitates recruitment of fast-spiking interneurons in the dentate gyrus. *J Neurosci*, 30, 11826-37.

- SANCHEZ-VIVES, M. V., BARBERO-CASTILLO, A., PEREZ-ZABALZA, M. & REIG, R. 2021. GABAB receptors: modulation of thalamocortical dynamics and synaptic plasticity. *Neuroscience*, 456, 131-142.
- SANCHO, L. & BLOODGOOD, B. L. 2018. Functional Distinctions between Spine and Dendritic Synapses Made onto Parvalbumin-Positive Interneurons in Mouse Cortex. *Cell Reports*, 24, 2075-2087.
- SARIHI, A., JIANG, B., KOMAKI, A., SOHYA, K., YANAGAWA, Y. & TSUMOTO, T. 2008. Metabotropic Glutamate Receptor Type 5-Dependent Long-Term Potentiation of Excitatory Synapses on Fast-Spiking GABAergic Neurons in Mouse Visual Cortex. *Journal of Neuroscience*, 28, 1224-1235.
- SIMONS, D. J. & CARVELL, G. E. 1989. Thalamocortical response transformation in the rat vibrissa/barrel system. *J. Neurophysiol*, 61, 311-330.
- SPREKELER, H. 2017. Functional consequences of inhibitory plasticity: homeostasis, the excitation-inhibition balance and beyond. *Curr Opin Neurobiol*, 43, 198-203.
- STERIADE, M., DOSSI, R. C., PARÉ, D. & OAKSON, G. 1991. Fast oscillations (2040 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat. *PNAS*, 88.
- SUN, Q. Q., ZHANG, Z., SUN, J., NAIR, A. S., PETRUS, D. P. & ZHANG, C. 2014. Functional and structural specific roles of activity-driven BDNF within circuits formed by single spiny stellate neurons of the barrel cortex. *Front Cell Neurosci*, 8, 372.
- SZABADITS, E., CSEREP, C., SZONYI, A., FUKAZAWA, Y., SHIGEMOTO, R., WATANABE, M., ITOHARA, S., FREUND, T. F. & NYIRI, G. 2011. NMDA Receptors in Hippocampal GABAergic Synapses and Their Role in Nitric Oxide Signaling. *Journal of Neuroscience*, 31, 5893-5904.
- TABATA, T. & KANO, M. 2010. GABAB Receptor-Mediated Modulation of Metabotropic Glutamate Signaling and Synaptic Plasticity in Central Neurons. 58, 149-173.
- TERUNUMA, M., REVILLA-SANCHEZ, R., QUADROS, I. M., DENG, Q., DEEB, T. Z., LUMB, M., SICINSKI, P., HAYDON, P. G., PANGALOS, M. N. & MOSS, S. J. 2014. Postsynaptic GABAB receptor activity regulates excitatory neuronal architecture and spatial memory. *J Neurosci*, 34, 804-16.
- TOPOLNIK, L., AZZI, M., MORIN, F., KOUGIOUMOUTZAKIS, A. & LACAILLE, J. C. 2006. mGluR1/5 subtype-specific calcium signalling and induction of long-term potentiation in rat hippocampal oriens/alveus interneurons. *J Physiol*, 575, 115-31.

- TOPOLNIK, L., CONGAR, P. & LACAILLE, J. C. 2005. Differential regulation of metabotropic glutamate receptor- and AMPA receptor-mediated dendritic Ca²⁺ signals by presynaptic and postsynaptic activity in hippocampal interneurons. *J Neurosci*, 25, 990-1001.
- TREMBLAY, R., LEE, S. & RUDY, B. 2016. GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. *Neuron*, 91, 260-92.
- VOGELS, T. P., SPREKELER, H., ZENKE, F., CLOPATH, C. & GERSTNER, W. 2011. Inhibitory Plasticity Balances Excitation and Inhibition in Sensory Pathways and Memory Networks. *SCIENCE*, 334, 1569 - 1573.
- VRUWINK, M., SCHMIDT, H. H., WEINBERG, R. J. & BURETTE, A. 2001. Substance P and nitric oxide signaling in cerebral cortex: anatomical evidence for reciprocal signaling between two classes of interneurons. *J Comp Neurol*, 441, 288-301.
- XU, H., JEONG, H. Y., TREMBLAY, R. & RUDY, B. 2013. Neocortical somatostatin-expressing GABAergic interneurons disinhibit the thalamorecipient layer 4. *Neuron*, 77, 155-67.
- XUE, J.-G., MASUOKA, T., GONG, X.-D., CHEN, K.-S., YANAGAWA, Y., LAW, S. K. A. & KONISHI, S. 2011. NMDA receptor activation enhances inhibitory GABAergic transmission onto hippocampal pyramidal neurons via presynaptic and postsynaptic mechanisms. *J Neurophysiol*, 105, 2897-906.
- XUE, M., ATALLAH, B. V. & SCANZIANI, M. 2014. Equalizing Excitation-Inhibition Ratios across Visual Cortical Neurons. *Nature*, 511, 596 - 600.
- YANG, W., CARRASQUILLO, Y., HOOKS, B. M., NERBONNE, J. M. & BURKHALTER, A. 2013. Distinct Balance of Excitation and Inhibition in an Interareal Feedforward and Feedback Circuit of Mouse Visual Cortex. *Journal of Neuroscience*, 33, 17373-17384.
- YANG, W. & SUN, Q. Q. 2018. Circuit-specific and neuronal subcellular-wide E-I balance in cortical pyramidal cells. *Sci Rep*, 8, 3971.
- ZHANG, S., XU, M., KAMIGAKI, T., HOANG DO, J. P., CHANG, W. C., JENVAY, S., MIYAMICHI, K., LUO, L. & DAN, Y. 2014. Selective attention. Long-range and local circuits for top-down modulation of visual cortex processing. *Science*, 345, 660-5.